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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

X. THE EFFECTS OF SURFACE-VOLUME RATIO AND REDUCED PRESSURE ON THE FERMENTATION OF CARBOHYDRATES BY *AEROBACILLUS POLYMYXA* AND *AEROBACTER AEROGENES*[1]

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Abstract

The rate of fermentation of wheat mashes by *Aerobacillus polymyxa* was markedly increased by providing a high surface-area–volume ratio. Exposure to air or oxygen *per se* was not a controlling factor since an atmosphere of nitrogen gave the same effect. Inhibition of fermentation of shallow layers of mash by exposure to carbon dioxide suggested that escape of fermentation gases (mainly carbon dioxide) might be the major factor affecting the fermentation rate. Support has been given to this hypothesis by the marked increase in rate obtained when wheat mashes were fermented under reduced pressures.

Fermentation of 15% wheat mashes, normally requiring 72 to 96 hr., were complete in 48 hr. under 5 in. pressure (absolute). The fermentation rate was accelerated by decreasing pressures but was retarded slightly by increasing depths of mash. The butanediol–ethanol ratio became progressively lower with decreasing pressures, showing that such conditions favour ethanol formation.

Fermentation of sugar media by *Aerobacter aerogenes* was only mildly stimulated by reduced pressures. This treatment compares unfavourably with aeration as a means of increasing the fermentation rate of this organism.

Introduction

The significance of surface–volume relations in the fermentation of wheat mashes by *Aerobacillus polymyxa* was pointed out first by Katznelson (2). Shallow layers of whole wheat mashes fermented at a faster rate and gave higher diol–ethanol ratios than deep layers. In spite of the importance of this observation it was not feasible to carry out large scale *A. polymyxa* fermentations in shallow layers. It became necessary to determine the factor or factors governing the fermentation rate and ratio of products under different surface–volume conditions and to find means of applying these factors to fermentation of deep mashes by *A. polymyxa*. A less detailed investigation of a similar nature was also made of the *Aerobacter aerogenes* fermentation.

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Materials and Methods

In the experiments described in this paper, two strains of *Aerobacillus polymyxa*, N.R.C. C 4 (2) and C 3 (2) from our own collection, and *Aerobacter aerogenes* N.R.R.L. 199 were used.

For *Aerobacillus* fermentations 15% whole wheat mash containing 1% of calcium carbonate was used as medium. The medium for the *Aerobacter* fermentations consisted of 10% of dextrose, 0.09% of potassium monohydrogen phosphate, 0.09% of potassium dihydrogen phosphate, 0.025% of magnesium sulphate, and 0.2% of urea. The *Aerobacillus* inoculum was brought up in two 24-hr. stages on 5% whole-wheat–yeast-extract–calcium-carbonate; the medium for the *Aerobacter* inoculum was dextrose–corn-steep-liquor–calcium-carbonate. Inoculum equal to 3% of the volume of the mash was used. All fermentation media were incubated at 30° C. The methods of analyses for butanediol, ethanol, acetoin, and organic acids have been previously described (3).

Since the results in general with C 4 (2) and C 3 (2) were substantially the the same, only those obtained with C 4 (2) are presented.

Experimental Results

Effect of Various Surface–volume Relations on the A. polymyxa Fermentation

The first experiments were designed to confirm the observation by Katznelson that the rate of fermentation of wheat mashes increased with larger surface–volume relations. Three-hundred-millilitre lots of 15% whole wheat mash were fermented in 500, 1000, 2000, 3000, 4000, and 6000 ml. Erlenmeyer flasks, using *A. polymyxa*. Mashes were analysed for diol and ethanol at 48, 72, and 96 hr. The experiment was done in triplicate.

The results given in Table I show that the rate of fermentation was greatly increased by a high surface–volume ratio, thereby confirming the original observation by Katznelson. The progressive increase in the diol–ethanol ratio from deep to shallow layers showed also that the rate of diol formation was enhanced more than that of ethanol. It may be noted also that all fermentations were practically complete at 72 hr. with the exception of that of the deepest mash.

The results for ethanol showed an optimum rate of formation at an intermediate depth of mash. This indicates that some factor besides surface–volume condition is involved, and it is suggested that the rate is subject to two opposing effects, an acceleration due to decreasing depth of mash and some inhibiting factor. It has been pointed out in another paper (1) that oxygen retards ethanol formation by *A. polymyxa*, and perhaps this accounts for the inhibition observed in mashes where surface exposure was relatively large. The main purpose of the present investigation was to determine the nature of the acceleration factor in the formation of both ethanol and diol.

In the foregoing experiment, the use of equal volumes of mash in different sized flasks permitted the surface area and depth of mash to vary simul-

TABLE I

EFFECT OF FERMENTING 300 ML. LOTS OF WHOLE WHEAT MASH WITH *A. polymyxa* AT VARIOUS SURFACE–VOLUME RATIOS

Fermentation period, hr.	Surface, cm.²/Volume, ml.	Butanediol, %	Ethanol, %	Total products, %	Ratio
48	0.16	1.72	0.97	2.69	1.77
	0.49	2.42	1.25	3.67	1.93
	0.66	2.66	1.17	3.83	2.27
	0.86	2.85	1.22	4.07	2.34
	0.95	3.06	1.22	4.28	2.51
	1.32	3.19	1.17	4.36	2.73
72	0.16	2.19	1.18	3.27	1.85
	0.49	2.92	1.51	4.43	1.93
	0.66	3.14	1.37	4.51	2.29
	0.86	3.22	1.34	4.56	2.40
	0.95	3.26	1.19	4.45	2.74
	1.32	3.32	1.17	4.49	2.84
96	0.16	2.55	1.41	3.96	1.81
	0.49	3.11	1.56	4.67	1.99
	0.66	3.24	1.38	4.62	2.35
	0.86	3.28	1.31	4.59	2.50
	0.95	3.38	1.16	4.54	2.91
	1.32	3.37	1.09	4.46	3.09

taneously. By using 4-litre bottles instead of Erlenmeyer flasks, it was possible to keep the surface area constant and to vary the depth and volume. Fifteen per cent wheat mash was fermented at various depths in the bottles. Analyses for diol and ethanol were made as before at 24, 48, and 96 hr. The earlier period was included, as changes in the fermentation are most marked at this time; the 96 hr. analyses served to show the yield of total products at completion. It can be seen from the results in Table II that the rate of fermentation is closely related to mash depth and is greatly accelerated in shallow layers. It is clear that the beneficial effect was more marked on diol than on ethanol formation, but since surface area was held constant the effect of mash depth on ethanol was more pronounced than in the previous experiment.

The deeper the mash layer, and consequently the more anaerobic the conditions the higher was the final proportion of ethanol formed. The slight drop in ethanol formation between 48 and 96 hr. in the shallowest layer cannot be satisfactorily explained, but may be due to either loss by evaporation or conversion to organic acids. The fact that the theoretical yield of products was obtained in the shallow fermentations showed that there was no shift from fermentative to oxidative dissimilation.

The previous experiments were carried out in air and it remained to be shown whether or not air (oxygen) *per se* was a factor in increasing fermentation rate. The simplest means of throwing light on this point was to do the same experiment in an anaerobic atmosphere, e.g., nitrogen.

TABLE II

EFFECT OF FERMENTING WHOLE WHEAT MASH WITH *A. polymyxa* UNDER CONDITIONS OF CONSTANT SURFACE, 227 CM.², AND VARYING DEPTH

Fermentation period, hr.	Surface, cm.² / Depth, cm.	Butanediol, %	Ethanol, %	Total products, %	Ratio
24	344	1.44	0.57	2.01	2.53
	151	1.27	0.59	1.86	2.15
	87	0.85	0.44	1.29	1.93
	60	0.83	0.47	1.30	1.77
	43	0.76	0.41	1.17	1.85
48	344	3.02	1.35	4.37	2.24
	151	2.72	1.44	4.16	1.89
	87	2.07	1.09	3.16	1.90
	60	1.67	0.92	2.59	1.82
	43	1.50	0.83	2.33	1.81
96	344	3.29	1.26	4.55	2.61
	151	3.06	1.47	4.53	2.08
	87	2.85	1.75	4.60	1.63
	60	2.54	1.57	4.08	1.60
	43	2.10	1.13	3.23	1.86

The effect of nitrogen on the fermentation as compared with that of air was studied by fermenting 300-ml. lots of wheat mash in 500, 2000, and 6000 ml. flasks. Nitrogen was passed over the surface of the media. A set of control mashes was fermented under identical conditions except that they were allowed to stand in their own gaseous atmospheres. Analyses for diol and ethanol were made at 24, 48, and 72 hr. The results are shown in Table III. In so far as total products are concerned, fermentation in shallow layers is markedly increased by exposure to nitrogen as well as to air, but the distribution of diol and ethanol is different in the two gases. In general, air

TABLE III

EFFECT OF FERMENTING 300 ML. OF WHOLE WHEAT MASH WITH *A. polymyxa* UNDER AEROBIC AND ANAEROBIC CONDITIONS AT VARIOUS SURFACE–VOLUME RATIOS

Fermentation period, hr.	Surface, cm.² / Volume, ml.	Butanediol, %		Ethanol, %		Total products, %		Ratio	
		Air	Nitrogen	Air	Nitrogen	Air	Nitrogen	Air	Nitrogen
24	0.16	0.78	0.93	0.41	0.53	1.19	1.46	1.90	1.75
	0.66	1.53	1.36	0.65	0.83	2.18	2.19	2.35	1.62
	1.32	1.96	1.62	0.76	1.07	2.72	2.69	2.58	1.31
48	0.16	1.96	1.62	0.84	1.26	2.80	2.88	2.33	1.23
	0.66	2.75	2.54	1.38	1.77	4.13	4.31	2.00	1.44
	1.32	2.93	2.66	1.33	1.76	4.26	4.42	2.20	1.51
72	0.16	2.22	2.03	1.17	1.26	3.39	3.29	1.90	1.61
	0.66	2.93	2.54	1.39	1.77	4.32	4.31	2.11	1.44
	1.32	3.06	2.66	1.22	1.76	4.28	4.42	3.06	1.51

stimulated the production of diol more than did nitrogen, but the effect on ethanol formation was the reverse. It may be concluded, therefore, that the accelerated rate of fermentation in shallow layers is not related to exposure to air *per se* since a nitrogen atmosphere gives a similar effect. It seemed likely, therefore, that the accelerating factor must involve some characteristic that shallow layers of mash possess under both aerobic and anaerobic conditions. This suggested that large surface area and relatively small depth would provide for more rapid escape of the gases (carbon dioxide and hydrogen) produced during fermentation and that this escape of gases might be the factor affecting the rate.

If the hypothesis stated above is correct then exposure of mashes of varying depths to an atmosphere of the fermentation gases should give an approximately constant fermentation rate. Since carbon dioxide makes up the main bulk of these gases its effect on the fermentation rate in various depths of mash was investigated.

As previously described, 15% mash was fermented at varying depths in 4-litre bottles in a changing atmosphere of carbon dioxide. Mashes fermented in their own atmospheres provided controls. The results given in Table IV show that at all depths mash fermented at practically the same rate under carbon dioxide. The data for total products show also that in the shallowest layer of mash the rate of fermentation was slightly depressed compared to the rate in air.

TABLE IV

EFFECT OF CONSTANT SURFACE, 227 CM.², AND VARYING DEPTH ON THE FERMENTATION OF 15% WHOLE WHEAT MASHES BY *A. polymyxa* UNDER AIR AND CARBON DIOXIDE ATMOSPHERES

Fermentation period, hr.	Surface, cm.² / Depth, cm.	Butanediol, %		Ethanol, %		Total products, %		Ratio	
		Air	Carbon dioxide	Air	Carbon dioxide	Air	Carbon dioxide	Air	Carbon dioxide
24	151	1.21	0.90	0.65	0.46	1.86	1.36	1.86	1.96
	87	1.06	0.91	0.51	0.49	1.57	1.40	2.12	1.86
	43	0.90	0.91	0.53	0.50	1.43	1.40	1.70	1.82
48	151	2.57	2.08	1.28	1.07	3.85	3.15	2.01	1.94
	87	2.25	2.10	1.21	1.22	3.46	3.32	1.86	1.72
	43	1.94	2.10	1.12	1.24	3.16	3.34	1.73	1.69
72	151	3.07	2.74	1.61	1.39	4.68	4.13	1.91	1.96
	87	2.89	2.77	1.68	1.80	4.57	4.57	1.72	1.54
	43	2.62	2.76	1.63	1.81	4.25	4.57	1.61	1.52

These experiments indicate that rapid fermentation of wheat mashes in thin layers, or, more precisely, under conditions of large surface–volume ratio, is attributable to the rapid escape of carbon dioxide. It might be contended that under conditions of active fermentation any mash would be covered by carbon dioxide, which would impose the same conditions as employed in the above experiment. Quantitatively, however, the atmosphere above a fer-

menting mash exposed to air cannot be 100% carbon dioxide since some air will reach the mash surface by diffusion. It is obvious, then, that in mashes of small surface–volume ratio the concentration of carbon dioxide will be relatively high, in which case the conditions will approximate more closely those of the above experiment.

Since the ready escape of carbon dioxide from fermenting mashes appeared to be an important factor in accelerating the rate of fermentation, it seemed logical to suppose that decreasing the total gas pressure over the mash would help achieve this effect. Furthermore such treatment would be applicable to deep as well as to shallow mashes.

Effect of Reduced Pressure on Fermentation Rate

A 2000 ml. flask was fitted with a water-cooled reflux condenser, which in turn was connected to a vacuum pump through a −80° C. cold trap and a mercury manometer. One litre of 15% whole wheat mash, inoculated with *A. polymyxa*, was put in the flask and the pressure reduced to 5 in. of mercury absolute. As a control an identical fermentation was carried out at atmospheric pressure. No foaming difficulties were encountered under reduced pressure. Samples were removed from control and test flasks at 24, 36, 48, and 96 hr. and analysed for diol and ethanol. The results are given in Table V. The effect of reduced pressure was to increase greatly the rate of fermentation, and the formation of both diol and ethanol. The relatively low diol–ethanol ratio indicated that the effect was more pronounced on ethanol. This experiment offers substantial proof of the hypothesis that removal of carbon dioxide is a primary factor in increasing the fermentation rate.

TABLE V

THE EFFECT OF REDUCED PRESSURE ON RATE OF FERMENTATION OF WHEAT MASH BY *A. polymyxa*

Fermentation time, hr.	Pressure, in. Hg	Butanediol, %	Ethanol, %	Total products, %	Ratio
24	30	1.03	0.50	1.53	2.06
	5	2.00	1.44	3.44	1.39
36	30	1.55	0.79	2.34	1.96
	5	2.40	1.85	4.25	1.30
48	30	2.09	1.13	3.22	1.85
	5	2.45	1.94	4.39	1.26
96	30	3.00	1.49	4.49	2.02
	5	2.45	2.02	4.47	1.21

In a further experiment 2-litre lots of 15% wheat mash were fermented under 10 in. of mercury absolute pressure in 4-litre Pyrex bottles. Control mashes were fermented at atmospheric pressure under similar conditions. Analyses for diol, ethanol, and pH were made at 24, 48, 72, 96, and 120 hr. These intervals provided a measure of the rate of fermentation as well as an

indication as to whether or not total products are realized under reduced pressure treatments. The results of duplicate mashes are given in Table VI. Again it is apparent that reduced pressure markedly accelerated the fermentation rate, the reactions under these conditions being about 95% complete in 48 hr. as compared with 60% completion in the control fermentations. A theoretical yield of total products (diol + ethanol = 4.6%) was obtained,

TABLE VI

EFFECT OF REDUCED PRESSURE ON RATE OF FERMENTATION AND PRODUCT YIELD BY *A. polymyxa*

Fermentation time, hr.	Pressure, in. Hg.	Butanediol, %	Ethanol, %	Total products, %	Ratio	pH
24	30	0.85	0.51	1.36	1.67	6.17
	10	1.66	1.14	2.80	1.45	5.78
48	30	1.76	1.03	2.79	1.71	6.00
	10	2.44	1.92	4.36	1.27	5.80
72	30	2.47	1.52	3.99	1.62	5.95
	10	2.51	1.96	4.47	1.28	5.95
96	30	2.65	1.73	4.38	1.53	5.90
	10	2.52	2.02	4.45	1.25	5.97
120	30	2.76	1.78	4.54	1.55	5.85
	10	2.52	2.06	4.58	1.23	5.98

showing that reduced pressure did not interfere with normal fermentative dissimilation. The pH changes were also worthy of note. Under atmospheric conditions there was a normal progressive fall in pH throughout the course of the fermentation. On the other hand, under reduced pressure the pH of the mash dropped to a level of 5.78 in 24 hr. and thereafter rose progressively to 5.98 in 120 hr. This rise in pH suggested proteolytic breakdown of the wheat proteins to give ammonia, and also that such decomposition only occurs to an appreciable extent when the carbohydrate source is nearly all consumed.

Effect of Depth of Mash and Absolute Pressure on the Fermentation Rate

Since acceleration of the fermentation rate by reduced pressure seemed to be due largely to the removal of fermentation gases it was important to know the effectiveness of the treatment at various depths of mash. It appeared likely that the removal of gases would become more difficult with increasing depth. Within the limits imposed by laboratory equipment an investigation was made of the interaction of mash depth and pressure on the rate of fermentation.

Fifteen per cent whole wheat mashes were fermented with *A. polymyxa* in 4-litre Pyrex bottles fitted with reflux condensers as previously described. The mash depths were 1.5, 2.6, 4.8, 8.8, and 13.2 cm. The pressures selected for study were 20, 10, and 5 in. of mercury absolute. Controls consisted of similar mashes of the same depths fermented at atmospheric

pressure. The analyses for diol and ethanol were made at 24 hr. only, as previous experiments had shown that the effect of reduced pressure was most marked at that time. Since considerable difficulty was experienced in obtaining concordant results from one experiment to another, even in the controls, a series of five experiments with controls was carried out at each pressure. The average results of the analyses from all experiments judged to be satisfactory are given in Table VII.

TABLE VII

EFFECT OF REDUCED PRESSURE AT VARIOUS DEPTHS AT 24 HR. ON THE FERMENTATION OF WHEAT BY *A. polymyxa*

Depth, cm.	Butanediol, %				Ethanol, %				Total products, %				Diol–ethanol ratio			
	Pressure, in. Hg				Pressure, in. Hg				Pressure, in. Hg				Pressure, in. Hg			
	30	20	10	5	30	20	10	5	30	20	10	5	30	20	10	5
1.5	1.06	1.29	1.63	2.00	0.48	0.68	1.09	1.49	1.54	1.97	2.72	3.49	2.21	1.90	1.50	1.34
2.6	0.89	1.14	1.60	1.43	0.46	0.65	1.07	1.39	1.35	1.79	2.67	3.32	1.93	1.75	1.50	1.39
4.8	0.76	1.03	1.65	1.85	0.42	0.61	1.16	1.52	1.18	1.64	2.81	3.37	1.81	1.69	1.42	1.22
8.8	0.73	1.05	1.60	1.82	0.43	0.66	1.12	1.48	1.16	1.71	2.72	3.32	1.70	1.59	1.42	1.23
13.2	0.72	1.12	1.67	1.83	0.44	0.72	1.23	1.60	1.16	1.84	2.90	3.43	1.64	1.55	1.36	1.14

At all depths of mash there was a clear-cut effect of reduced pressure on the yields of diol and ethanol; both were markedly increased by progressive reduction in pressure. However, the effect was more marked on ethanol than on diol as shown by the progressive drop in the diol–ethanol ratio with decreasing pressure. The significance of these results was confirmed by statistical analysis. As shown before, under atmospheric pressure the overall effect of decreasing the mash depth was to increase the rate of formation of products, diol formation being accelerated more than that of ethanol. At 20 in. pressure the effect of depth on diol formation was similar to that at atmospheric pressure, with the exception of the deepest mash. At the lower pressures the effect of depth was less marked owing perhaps to the more effective removal of carbon dioxide. The increased production of ethanol in deep mashes had been shown earlier to be related to the degree of anaerobiosis; lower pressures accentuate this condition by decreasing the oxygen content of the gas atmosphere.

At all three pressures below atmospheric the minimum yield of total products occurred at some depth intermediate between the two extremes. This effect applied to both diol and ethanol, and may have been due to a mixing phenomenon that became effective at reduced pressures. It was noted that bubbles of gas floated bran and other solid particles of the media to the surface where gas release permitted the solids to drop back slowly to the bottom of the flask. In this way, a steady and thorough mixing of the mash took place, which appeared to aid considerably in the evolution of gas. Since this mixing was more pronounced in deep mashes the net effect was to partially neutralize

the acceleration normally observed in shallow mashes. The mash depths used in these experiments were chosen arbitrarily and because of experimental conditions were not great compared to those found in large scale operations. It seems reasonable to suppose that if depth of mash were increased greatly it would eventually become a dominant factor in the release of fermentation gases. Under such conditions agitation might be combined with reduced pressure to advantage.

Effect of Reduced Pressure on the Fermentation of Glucose by Aerobacter aerogenes

In view of the foregoing results, which showed an accelerating effect of reduced pressure on the rate of fermentation of wheat mash by *Aerobacillus polymyxa*, it seemed worthwhile to subject *Aerobacter aerogenes* fermentations to the same treatment. The culture media used in this experiment has been described earlier in the paper. Quantities of media were put in 4-litre bottles to give depths of 1.5, 2.6, 4.8, 8.8, and 13.2 cm., respectively. The bottles were fitted with water-cooled reflux condensers and connected to a vacuum pump as before. Absolute pressures of 20, 10, and 5 in. of mercury were used in the experiment. Controls for each reduced pressure experiment were provided by similar media fermented at atmospheric pressure. Analyses were made for butanediol, ethanol, and acetoin at the end of a 24 hr. fermentation period. The diol figure used in calculating the diol–ethanol ratio included acetoin. Results are given in Table VIII.

TABLE VIII

EFFECT OF REDUCED PRESSURE, AT 24 HR., ON THE FERMENTATION OF GLUCOSE AT VARIOUS DEPTHS BY *Aerobacter Aerogenes* N.R.R.L. 199

Depth of media, cm.	Butanediol, %				Ethanol, %				Acetoin, %				Diol–ethanol ratio			
	Pressure, in. Hg				Pressure, in. Hg				Pressure, in. Hg				Pressure, in. Hg			
	30	20	10	5	30	20	10	5	30	20	10	5	30	20	10	5
1.5	0.36	0.50	0.58	0.61	0.24	0.32	0.37	0.36	0.04	0.06	0.05	0.04	1.50	1.56	1.57	1.69
2.6	0.31	0.47	0.54	0.68	0.21	0.26	0.36	0.39	0.03	0.06	0.06	0.06	1.47	1.80	1.50	1.77
4.8	0.30	0.36	0.46	0.74	0.20	0.25	0.34	0.43	0.03	0.06	0.05	0.06	1.50	1.44	1.35	1.72
8.8	0.29	0.35	0.56	1.21	0.19	0.19	0.37	0.69	0.02	0.05	0.05	0.10	1.52	1.54	1.51	1.76
13.2	0.29	0.32	0.60	1.36	0.18	0.21	0.37	0.78	0.02	0.04	0.06	0.10	1.61	1.52	1.62	1.75

Increasing reduction of pressure produced a progressive increase in yields of both diol and ethanol in all depths of medium. The effect of increasing depth at atmospheric and 20 in. pressure was to decrease the rate of fermentation. Ten inches pressure at an intermediate depth gave the minimum rate, while at the lowest pressure (5 in.) the deep mashes fermented much more rapidly.

These results parallel, in general, those obtained in the *Aerobacillus polymyxa* fermentations, but there is one outstanding difference, namely, the reversed effect of depth at the lowest pressure. Apart from inherent differences in

the two types of organism, this effect may be attributed to the much lower viscosity of the glucose medium, which permitted greater mixing and more ready escape of gas with increasing depth.

Discussion

Provision for the rapid removal of fermentation gases from whole wheat mash increased the rate of fermentation by *A. polymyxa*. Further confirmation was provided in previous work by aeration of fermenting media with various gases (1). Although oxygen and air specifically stimulated diol formation, and nitrogen and hydrogen had a similar effect on ethanol production, the over-all effect of aeration was to increase the rate of fermentation. The effect common to all the aeration treatments was the mechanical removal or sweeping out of the carbon dioxide produced by fermentation. The fact that aeration produced the same general effect as reduced pressure indicated that the latter treatment did not bring about any special metabolic activity of the organism.

It was noted during the course of the present experiments with reduced pressure as well as in previous investigations involving aeration that all treatments caused a marked increase in bacterial numbers. Although this was to be expected with air and oxygen it was not anticipated with nitrogen and hydrogen nor with the reduced pressure treatments. The increased fermentation rate might be explained on the basis of greater numbers of bacteria although as yet no satisfactory correlation between numbers and products yield has been established. Results of further investigations on this point will be published shortly.

The effect of reduced pressure on *Aerobacter* fermentations was not as marked as on *Aerobacillus* as shown by the yield of total products in 24 hr. It is known, however, that rapid fermentation with *Aerobacter* cannot be achieved without vigorous aeration; normally this is accomplished with air but it has been shown that nitrogen is equally effective (1). This observation was interpreted as meaning that removal of carbon dioxide by the aerating gas was the main factor influencing rate of fermentation. However, a similar removal of gas by reduced pressure and the establishment of anaerobic conditions (as under nitrogen) failed to give the same stimulation to fermentation, especially in the shallower layers of media. Only at the lowest pressure and in the deepest media did the *Aerobacter* fermentation give markedly increased yields.

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PACKAGING

III. EFFECT OF MOULD GROWTH AND AGEING ON THE WATER-VAPOUR TRANSMISSION OF PACKAGING MATERIALS[1]

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Abstract

Packaging materials were dusted with mould spores and stored in a cabinet at 95° F. and 95 to 100% relative humidity for periods of one to eight weeks.

M.S.T. and M.S.A.T. "Cellophane" were attacked only slightly by mould, but deterioration of the heat-sealing, moisture-proof lacquer occurred during storage under conditions suitable for optimum mould growth. Moulds grew abundantly on M.S.Y.T. Cellophane. Wax-coated materials supported abundant mould growth, and their water-vapour transmission values increased when wax peeled from the surface of the sheet. The transmission rate of laminated materials having metal foil as one layer was not greatly affected by mould growth or delamination of the other layers. Abundant mould growth developed on most samples of kraft, and on glassine. Very little mould developed on cellulose acetate, Pliofilm, or vinyl-film.

Introduction

The effect of mould growth is undoubtedly an important factor in the deterioration of packaging materials if the atmosphere surrounding the package is of high humidity, or if the package contains material of high moisture content, i.e., material likely to be in equilibrium with high humidities. Concurrently with exposure to conditions favourable to the growth of moulds, changes may occur in packaging materials such as weakening of the fibre structure, delamination, or separation of a coating from the base material. Hence, the present investigation was undertaken to assess the effects of mould growth and ageing on the water-vapour transmission of a variety of packaging materials.

Materials and Methods

The materials used were various combinations of kraft paper, metal foil, glassine, "Cellophane," cellulose acetate, vinyl-film, and Pliofilm, both waxed and unwaxed. In addition, different grades and plies of Cellophane were investigated. When samples of duplex or triplex Cellophane were tested, the layers were heat sealed around the edge of the samples since these materials would be sealed in this manner when in use. Details of materials are noted in Table I.

The initial water-vapour transmission of the materials was determined. Samples were then dusted with mould spores of a variety of species and placed in a mould infested cabinet, operating at 95° F. and at 95 to 100% relative

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[3] *Mycologist, Industrial Utilizations.*

humidity. Species included in the inoculum were; *Syncephalastrum racemosum*, *Paecilomyces variota*, *Penicillium* spp., *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Chaetomium globosum*, and *Stachybotrys atra*. Samples were removed after storage for one, two, four, and eight weeks, were inspected for mould growth and physical deterioration, and the water-vapour transmission determined. All measurements of water-vapour transmission were made using a vapometer at a vapour pressure differential of approximately 26 mm. of mercury and a dry bulb temperature of 80° F. The vapometer was the same as that used in a previous study of packaging materials (2). It consisted of a flanged aluminium cup containing 10 ml. of water. Over the mouth of the cup the sample could be attached by another flange securely bolted to effect the seal. Although more accurate methods of measurement are desirable (1), this technique was satisfactory for the present purpose and provided a relative scale for comparing changes in water-vapour transmission occasioned by mould growth and ageing.

Results

The results are shown in Figs. 1 to 20, in which the figure numbers correspond with the material numbers given in Table I. All water-vapour transmissions plotted are averages of triplicate determinations. The severity of attack by moulds was classified into four groups: none (*N*), surface free of mould growth; slight (*S*), growth had started at particles of foreign matter on the surface of the material; medium (*M*), growth was more general and moulds were actually feeding on the packaging material; abundant (*A*), growth was heavy and general.

M.S.T. and M.S.A.T. Cellophane were only slightly attacked by moulds except in spots where the lacquer had been damaged by heat sealing (Figs. 1, 2, 3, and 5). It is believed that the fairly large increase in the water-vapour transmission of these materials was due to loosening and cracking of the heat-sealing lacquer brought about by the severe conditions of storage, rather than to mould growth. In contrast to the above grades, M.S.Y.T. Cellophane (Fig. 4) developed an abundant growth of mould over its entire surface. Possibly a difference in the processing of this type of film caused this greater proliferation of organisms. There was a much smaller increase in the transmission of duplex and triplex Cellophanes (Figs. 2, 3, 4, and 5) than in that of the single sheet (Fig. 1). This probably occurred because some protection was afforded the lacquer on the inner faces of the Cellophane.

Abundant mould growth occurred on all materials that were wax-impregnated or wax-coated (Figs. 6 to 10). Wax-impregnated kraft (Fig. 6) showed a large increase in water-vapour transmission concurrent with abundant mould development. Wax-coated kraft (Fig. 7) suffered only a slight increase in water-vapour transmission, and only a slight separation of the wax from the kraft. Wax-coated Cellophanes (Figs. 8 and 9), however, showed large increases in their water-vapour transmission, and the wax tended to peel from the Cellophane. The increased transmission values of wax-coated

TABLE I

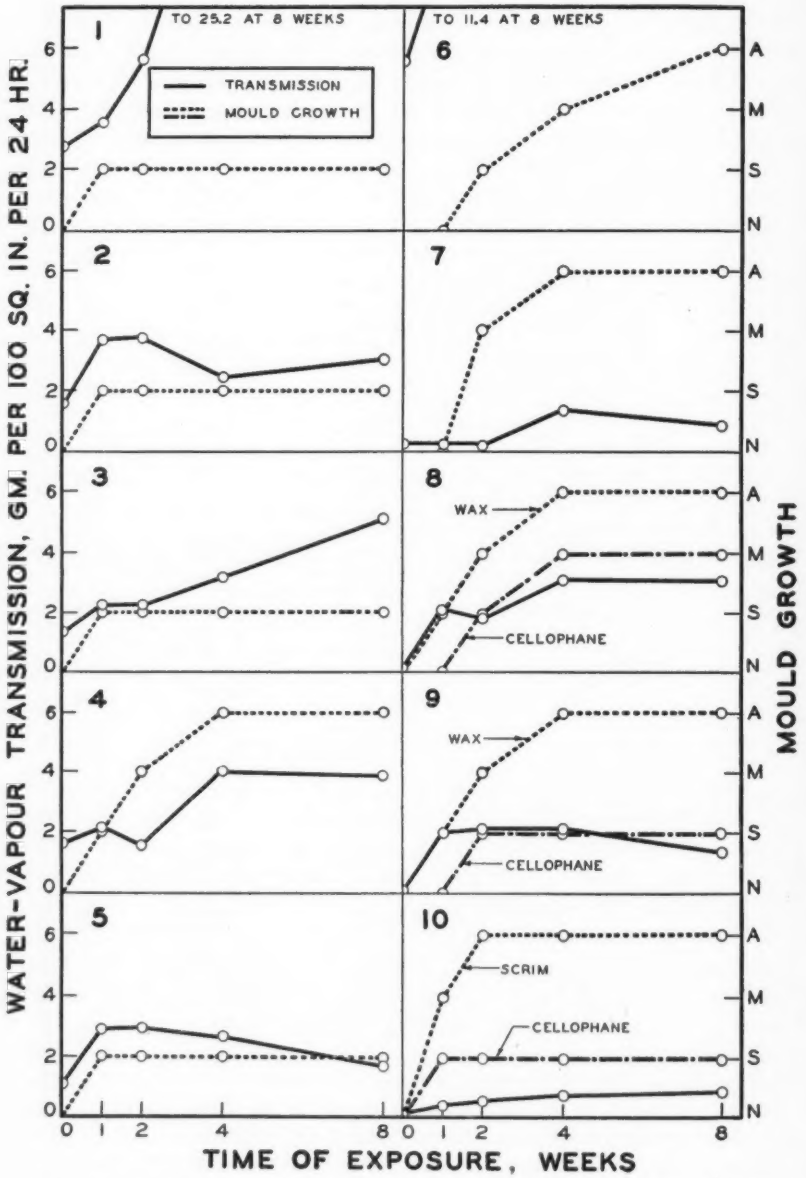
PACKAGING MATERIALS STUDIED

Material No.	Description of material
	Cellophanes
1	300 M.S.A.T., single
2	300 M.S.T., duplex
3	300 M.S.A.T., duplex
4	300 M.S.Y.T., duplex
5	300 M.S.A.T., triplex
	Waxed materials
6	40 Lb. kraft, wax impregnated
7	40 Lb. wet strength kraft, wax coated* 40 lb./ream
8	300 M.S.T. Cellophane, wax coated* 40 lb./ream
9	300 M.S.T. Cellophane laminated to 300 M.S.T. Cellophane, wax coated* 40 lb./ream
10	300 M.S.A.T. Cellophane laminated to scrim. Cellophane lightly waxed, scrim heavily waxed*
	Laminated materials
11	300 M.S.T. Cellophane laminated to 300 M.S.T. Cellophane
12	300 M.S.T. Cellophane laminated to metal foil
13	300 M.S.A.T. Cellophane laminated to 25 lb. kraft.
14	40 Lb. kraft laminated to alloyed lead foil laminated to 300 M.S.T. Cellophane
15	Scrim laminated to kraft laminated to alloyed lead foil with butvar coating
16	25 Lb. kraft laminated to cellulose acetate
17	25 Lb. kraft laminated to 25 lb. bleached glassine
18	25 Lb. bleached glassine laminated to 25 lb. bleached glassine
19	Vinyl-film laminated to both sides of 0.001 in. aluminium foil
	Pliofilm
20	Pliofilm, single

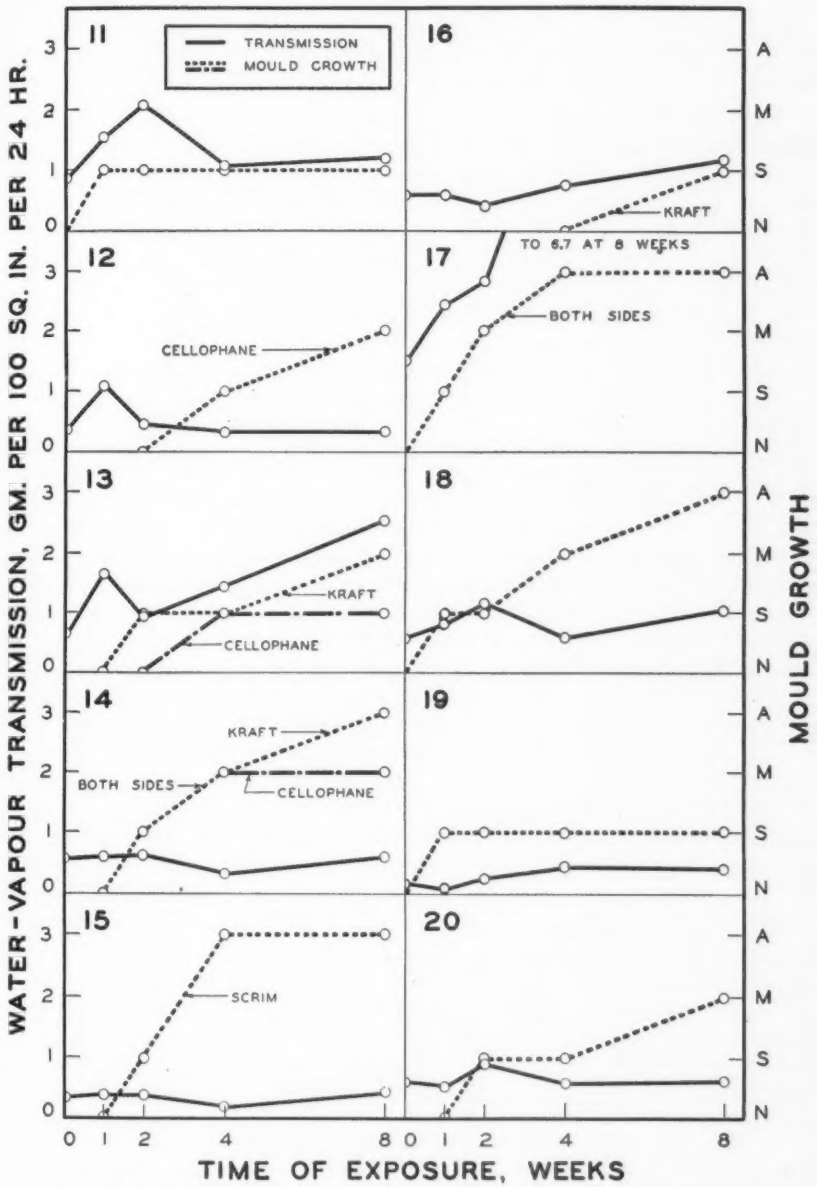
* *Flexible wax compound.*

materials were probably the result of this loosening of the wax coating. The data show the extent of the reduction in water-vapour transmission resulting from wax application, and its subsequent increase with separation of the wax from the base stock. Scrim laminated to Cellophane and heavily waxed (Fig. 10) supported abundant mould growth and showed a gradual but consistent increase in water-vapour transmission.

The water-vapour transmission of laminated materials having metal foil as one layer was not greatly affected even though moulds grew abundantly on the other layers and considerable delamination occurred. Examples of this were: Cellophane laminated to metal foil (Fig. 12), where considerable delamination occurred, and kraft laminated to foil laminated to Cellophane (Fig. 14), where considerable delamination of the Cellophane and slight delamination of the kraft occurred. Also, the foil blistered slightly on samples of foil laminated to kraft backed with scrim (Fig. 15). Very little mould grew on vinyl-film laminated to aluminium foil (Fig. 19) and very slight delamination occurred. This very low increase in water-vapour transmission indicated the general desirability of using a foil type barrier when



FIGS. 1-10. *Effect of mould growth and ageing on the water-vapour transmission of Cellophane and of waxed packaging materials. (See Table I for key).*



FIGS. 11-20. *Effect of mould growth and ageing on the water-vapour transmission of laminated packaging materials and of Pliofilm. (See Table I for key).*

severe conditions of temperature and humidity are likely to be encountered. Equal protection might not be provided, however, in a completed package, since the barrier would be of little value if the material forming the heat-sealing surface loosened from the foil.

Most of the materials having kraft paper as an outer layer developed abundant mould growth on the kraft side. In addition to supporting a heavy growth of moulds on the kraft side, samples of Cellophane laminated to kraft (Fig. 13) delaminated considerably and the water-vapour transmission showed a significant increase. No mould grew on the cellulose acetate sheet laminated to kraft (Fig. 16), and only a slight amount on the kraft. The acetate sheet appears to have inhibited growth on the kraft. Moulds grew abundantly on kraft laminated to glassine (Fig. 17) and caused marked deterioration, penetrating the sheet completely at one point. Laminated glassine itself (Fig. 18) supported abundant mould growth, but the increase in water-vapour transmission was much less than that of glassine laminated to kraft.

Pliofilm (Fig. 20) showed only slight mould growth, and little increase in water-vapour transmission.

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DRIED MILK POWDER

V. THE PHOTOLYSIS OF RIBOFLAVIN IN MILK POWDERS[1]

By W. A. Bryce[2]

Abstract

Exposure of milk powders to sunlight resulted in a much greater destruction of riboflavin than did exposure to ultra-violet light in the range 3200 to 4200 Å. The rate of photolysis was greater for skim-milk powders than for whole milk powders. Increased intensities of visible light accelerated riboflavin destruction. In the spectral region of 4200 to 5600 Å the wave band causing the greatest destruction in liquid skim-milk had a principal wave-length of 4450 Å, which corresponded to a maximum in the absorption spectrum of riboflavin. The rate of photolysis of riboflavin was a function of both wave-length and intensity of the impinging energy.

Introduction

Riboflavin, or vitamin B_2, is a valuable component of foodstuffs. It is effective in promoting growth in rats, and in preventing lesions of cheilosis and nutritional anemia in humans (11). It is also an important factor in cell respiration and metabolism (11). It is stable to heat, but is readily decomposed when exposed to light (4). Since milk is an important source of riboflavin, the photochemical decomposition, or photolysis, of this vitamin in milk has been studied. Liquid whole milk in commercial milk bottles exposed to summer sunlight for two hours lost two-thirds of its riboflavin (6, 9, 15). Sunlight apparently causes much greater destruction of riboflavin than ultra-violet light, as it has been reported that commercial irradiation of milk sufficient to produce a vitamin D content of 400 U.S.P. units per quart had no effect on the riboflavin content (3). Another investigator reported a slight decrease in riboflavin content of milk as a result of commercial irradiation (16).

Increases in either alkalinity or temperature accelerate the photolysis of riboflavin in liquid whole milk. Temperature and pH are believed to affect the photodecomposition rather than other chemical decompositions, for when riboflavin solutions are heated in the dark at 100° C. under mild acid conditions for four hours, little or no destruction of the vitamin occurs (14).

Since milk powder may be exposed to light during post-drying handling and storage and while it is being prepared for consumption, it was felt that some information on the photolysis of riboflavin in milk powders would be of value. This paper describes an investigation designed to study the effect of sunlight and ultra-violet light on the riboflavin content of milk powders of various fat contents obtained from different plants. It also includes some incidental measurements on riboflavin destruction in liquid skim-milk and on light absorption by riboflavin.

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Materials and Methods

The powders used in the study were the commercial spray-dried products of two Canadian companies. Powders of 1, 26, 28, and 30% butter fat were obtained from one plant, and of 1, 26, and 28% butter fat from another, all powders having a moisture content of 3.0%.

All riboflavin determinations were made using the photofluorometric method developed by Hodson and Norris (5). After the initial riboflavin contents were determined, samples of the powders were placed in sealed Pyrex glass containers, and were analysed for riboflavin content after exposure to sunlight for 60, 110, and 215 hr. (the latter period corresponding to 48 days' storage) at an average temperature of 6.1° C. (43° F.). The average light energy falling on the powders was assumed to be 1.8 cal. per sq. cm. per min. During the study the containers were shaken by hand every three days in an attempt to ensure that all of the powder would be exposed. Control samples of the powders were stored in light-proof containers at the same temperature.

Additional samples of the same powders were exposed in Pyrex glass containers at a distance of 6 in. from a 250 watt Purple-X bulb that emitted ultra-violet light in the range 3200 to 4200 Å. The energy falling on the samples was calculated to be approximately 0.0034 cal. per sq. cm. per min. Riboflavin determinations were made after periods of 75, 150, and 300 hr. (42 days' storage). The temperature during this phase of the study was 38° C. (100° F.). The powders were shaken after each 10 hr. of exposure. Control samples in light-proof containers were stored under the same conditions.

The effect of variations in 'light' intensity on the rate of photolysis of riboflavin in milk powders was also studied, as calculations on the energies falling on the samples from the sun and from the ultra-violet bulb showed great differences between the two sources. Samples of whole and skim-milk powders were sealed in flat glass containers, and were exposed for 75 hr. at distances of 1 ft. and 4 ft. from a 100 watt tungsten lamp. The energy intensities at the two levels were 0.0036 and 0.0004 cal. per sq. cm. per min., respectively. The destruction of the riboflavin at each intensity of light was determined.

It was recognized that the riboflavin destruction caused by different parts of the near ultra-violet and visible spectrum might not be equal, and therefore an attempt was made to determine which bands were the most destructive. Samples of fresh skim-milk in glass cuvettes were exposed to successive wave bands from 4200 to 5600 Å, all bands being 200 Å wide and of equal intensity. The bands were obtained by the use of Corning glass filters. The intensities, measured by means of a Weston exposure meter, were adjusted to a constant value by varying the potential applied to the tungsten lamp used as the light source. After exposure for four hours the riboflavin content of the milk samples was determined.

The absorption spectrum of riboflavin in aqueous solution was also determined for the above light range, as it was believed that a direct relation

should exist between the wave-lengths causing maximum destruction and those at which the greatest absorption occurred. A Beckman Quartz Spectrophotometer was used to determine the absorption spectrum.

Control samples of powders in light-proof containers were maintained under comparable storage conditions whenever powders were exposed to visible or ultra-violet light. No decrease in riboflavin content occurred in any of these control samples during storage.

Results

The data for whole and for skim-milk powders were assessed by analysis of variance. No difference was observed between samples of whole or between samples of skim powders exposed to sunlight. The time of exposure had an effect on the whole milk powders but any change in skim powders was not assessed as significant. The results for samples exposed to sunlight are shown in Fig. 1. Marked destruction of the riboflavin occurred during the first 60

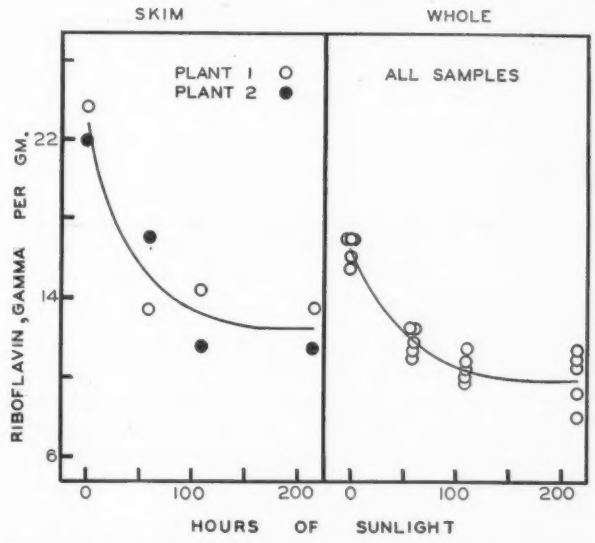

FIG. 1. *The effect of sunlight on the destruction of riboflavin in milk powders from Plants 1 and 2.*

hr., after which the rate of photolysis was greatly reduced. Skim-milk powders had higher riboflavin contents than whole milk powders and suffered greater destruction than the whole powders. Vitamin losses occurred at approximately equivalent rates in the skim-milk powders from the two plants, the initial differences being largely maintained throughout.

Similar mathematical assessment of the data for powders exposed to ultra-violet light showed that the only significant difference was that between skim-milk powders from the two producers. Nevertheless, there was some evidence

of riboflavin destruction (Fig. 2). The rate of destruction was about the same for both types of powders, since the curves for the skim and whole milk powders were practically parallel. The most pronounced difference in these comparisons was that resulting from exposure to the two different light sources. Sunlight caused a greater decrease in riboflavin content than ultra-violet light.

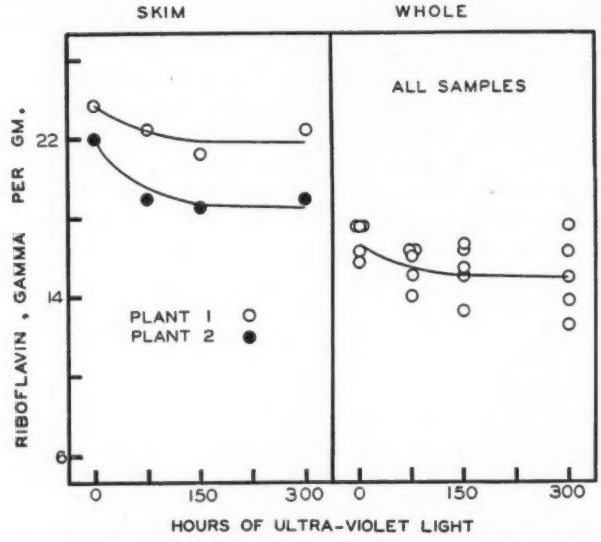

FIG. 2. *The effect of ultra-violet light (3200 to 4200 Å) on the destruction of riboflavin in milk powders from Plants 1 and 2.*

Variations in the intensity of the energy falling on the samples had a marked effect on the rate of photolysis of the riboflavin in skim-milk powders as is evident from the data in Table I and from a comparison of Figs. 1 and 2. In whole milk powders, the differences in energy did not greatly affect the rate of destruction of the riboflavin.

TABLE I

EFFECT OF VARIATION IN INTENSITY OF LIGHT ON DESTRUCTION OF RIBOFLAVIN; 75 HR. EXPOSURE TO A 100 WATT TUNGSTEN LAMP

Distance from lamp, ft.	Energy level, cal./sq. cm./min.	Riboflavin content (gamma/gm.)			
		Skim-milk powder		Whole milk powder	
		Initial	Final	Initial	Final
1	0.0036	22.00	12.50	17.50	10.00
4	0.0004	22.00	17.00	17.50	11.75

The data for the comparison of the destructive effect of wave bands between 4200 and 5600 Å are shown in Fig. 3. While all wave bands studied caused some destruction of the riboflavin, the greatest decrease occurred at the band having a principal wave-length of 4450 Å. From Fig. 4 it can be seen that this wave-length corresponds approximately to a maximum in the absorption spectrum of riboflavin.

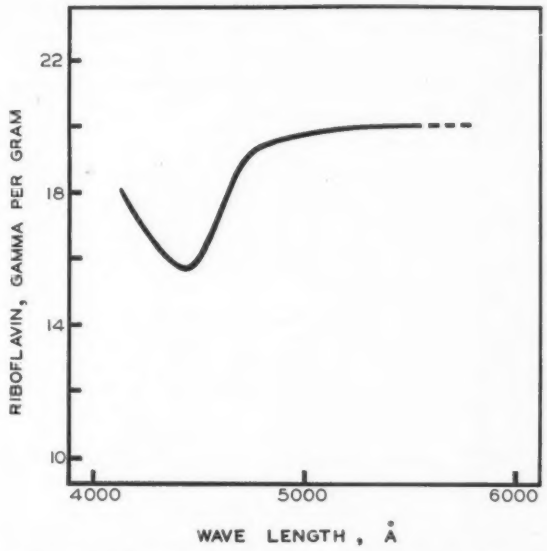

FIG. 3. *The effect of various wave-lengths of light on the destruction of riboflavin in liquid skim-milk. (Riboflavin content calculated on basis of milk solids; initial value, 22 gamma per gm.).*

Discussion

The destruction of riboflavin in the milk powders was much more rapid and complete owing to exposure to sunlight than it was as a result of exposure to ultra-violet light. This was believed to be due to the fact that the energy in the sunlight was much greater than that from the ultra-violet source, and also because the most destructive wave-lengths were not present in the ultra-violet light used in this experiment. The data in Table I showed that the greatest destruction occurred in the powders at the greatest level of light intensity. The effect was much more noticeable for skim-milk powders than it was for whole milk powders.

The wave-length of the incident light was an important factor in the photolysis of riboflavin. The marked destruction of the vitamin due to sunlight action was probably partially attributable to the presence in sunlight of the destructive wave band around 4450 Å. Further evidence of the significance of wave-length of the impinging light on the rate of riboflavin photolysis was

provided by the observation that when skim-milk powder was exposed to visible light and to ultra-violet light of equal intensity (0.0034 cal. per sq. cm. per min.) for the same length of time (75 hr.), 43% of the riboflavin was destroyed by the visible light, and only 10% was destroyed by the ultra-violet light. Only in the visible light was the destructive wave band around 4450 Å present. The fact that commercial irradiation of milk causes only a slight decrease in riboflavin content is possibly due to the absence of these destructive wave-lengths from the ultra-violet light used.

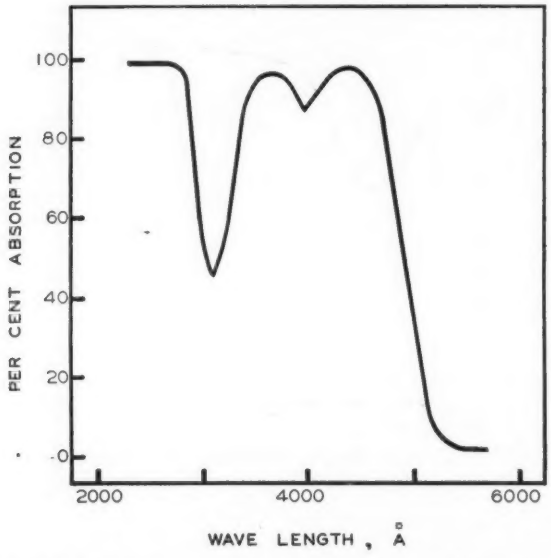

FIG 4. *The absorption spectrum of riboflavin in the range 2500 to 5500 Å.*

The apparent decrease in the rate of destruction of riboflavin in the milk powders after prolonged exposure to sunlight has also been observed during the photolysis of alkaline solutions of pure riboflavin*. In the latter study, the results obtained from photofluorometric assays were not confirmed by microbiological determinations. Materials with chemical and fluorescent properties similar to those of riboflavin may have been measured as riboflavin in the photofluorometric analysis. Irradiation products of riboflavin have been isolated and identified (7, 8) and the fluorescent spectrum of one of these substances, lumiflavin, is similar to that of the vitamin (1, 2, 7, 13).

The decrease in rate of photolysis may also be attributed to the formation of substances that protected the vitamin from further photolysis. This problem is receiving attention in these laboratories at the present time.

* *Gorham, P. Unpublished results.*

Acknowledgments

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LIQUID AND FROZEN EGG

I. APPLICATION OF A FLUORESCENCE MEASUREMENT TO LIQUID AND FROZEN EGG[1]

By Margaret Reid[2]

Abstract

The direct measurement of fluorescence on clarified sera collected as exudate from defrosting egg distinguished between samples of frozen egg of varying quality. The method may be useful in egg drying establishments where large quantities of frozen egg are available for test purposes.

A similar serum was extracted from liquid and frozen egg by the following procedure. Approximately 50 ml. of fresh or thawed egg liquid was added to approximately 100 ml. of chloroform, mixed by gentle swirling, allowed to stand two minutes, shaken vigorously for 30 sec., and immediately centrifuged for 15 min. at 2000 r.p.m. The top layer, consisting of 15 to 20 ml. of red serum, was poured off and clarified by centrifuging for one minute, and the fluorescence of the clear serum determined. The latter measure consistently distinguished between samples of liquid and frozen egg of varying quality. The serum produced was less highly fluorescent than that obtained as exudate, and consequently the range between quality types was narrowed.

The sera extracted with chloroform from freshly broken eggs of good quality had a higher fluorescence than that extracted from the material after standing at temperatures of 30° to 90° F. for short periods. However, the fluorescence rose when deterioration became evident. Freezing of liquid egg resulted in a decrease in fluorescent materials.

Introduction

The seasonal production of eggs, coupled with large wartime commitments, has made it necessary to store unprecedented quantities in the form of frozen blocks. No suitable test has been available for estimating the quality of liquid or frozen egg. During a preliminary investigation the following methods were tried without success: reducing sugar values measured on a tungstic acid filtrate (5, pp. 416 and 438); acid-soluble phosphorus (5, p. 461); peroxidase value (3); the fluorescence (6) of tungstic acid and trichloracetic acid filtrates of egg melange (5, pp. 416 and 461); pH (8); fluorescence of the liquid egg, a modification of a technique applied to egg powder (4); loaf volume (7); foaming volume (7); and solids content (1). Palatability ratings on the frozen egg liquid, cooked as scrambled egg and scored by the methods used for reconstituted dried egg powder (6), were also of little value in distinguishing quality. Eggs frozen from material discarded as infertile after 7 to 10 days' incubation were frequently preferred to a Grade *A* product.

During the course of these investigations, it was observed that a pink watery serum separated from frozen egg while defrosting. A similar serum could be extracted from both the fresh and defrosted products by shaking with chloroform and centrifuging. Measurements of refractive index, pH,

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[2] *Biochemist, Food Investigations.*

reducing sugar, and fluorescence were made directly on this extracted serum. Of these, only fluorescence showed promise as a possible measure of quality. The present paper describes the results to date of applying the fluorescence test to sera from samples of liquid and frozen egg of varying quality.

Materials and Methods

Materials

The various qualities of liquid and frozen egg used are described in the tables.

Methods

In preliminary work, serum was obtained by collecting the exudate from thawing egg. This material was clarified by centrifuging for one minute at 2000 r.p.m., and a fluorescence measurement was made on the supernatant fluid by methods previously described (6). However, 4 to 6 lb. of frozen egg was required to produce sufficient serum, and, moreover, no serum separated from egg frozen and stored for less than a month.

Separation of a similar serum from all types of liquid and frozen egg was brought about by adding chloroform to the egg, shaking, and centrifuging. In the adapted procedure, approximately 50 ml. of fresh or thawed egg liquid, which had been well mixed and strained, was added to approximately 100 ml. of chloroform, mixed by gentle swirling to avoid emulsification, allowed to stand for two minutes, shaken vigorously for 30 sec., and immediately centrifuged for 15 min. at 2000 r.p.m. The top layer, consisting of 15 to 20 ml. of red serum, was poured off and clarified by centrifuging for one minute. The fluorescence of the clear serum was then determined.

Results

The results are given in Tables I to IV, in which the samples are listed in order of decreasing quality.

In Table I the fluorescence values of sera obtained by seepage are compared with those of sera obtained by the chloroform extraction of materials representing a wide range of quality. It will be noted that both methods showed an increase in fluorescence values as quality decreased. Fluorescence values for sera obtained by seepage were consistently higher than the values for chloroform-extracted sera. Chloroform extraction appeared to remove approximately one-third of the fluorescing materials. The higher fluorescence values for sera collected by seepage resulted in better differentiation between quality types; nevertheless the chloroform extraction procedure was used in the subsequent work reported here because of its economy of time and materials.

Table II shows the effect of storing egg liquid at temperatures ranging from 30° to 90° F. Although fluorescence of the sera increased with increasing storage temperature, storage at the lower temperatures apparently resulted in an initial decrease in fluorescing materials. This behaviour was supported

TABLE I

FLUORESCENCE OF SERA OBTAINED FROM FROZEN EGG AFTER THREE MONTHS' STORAGE

(Means of six measurements)

Description of sample	Fluorescence	
	Sera extracted with chloroform	Sera obtained by seepage
Grade *A* (no off-odour)	6.9	9.4
Grade *C* (definite off-odour)	7.6	11.9
Grade *C* held in shell at room temperature for three weeks prior to freezing (definite off-odour)	8.3	17.5
Incubator rejects* (strong off-odour)	21.4	33.0
Grade *A* broken out and held at 80° F. for 36 hr. prior to freezing (extreme off-odour)	38.8	43.7

* *Infertile eggs rejected during incubation.*

TABLE II

EFFECT OF TEMPERATURES ABOVE FREEZING ON FLUORESCENCE OF SERA EXTRACTED FROM LIQUID EGG STORED FOR 32 HR.

(Means of two measurements)

Description of sample	Fluorescence of serum
Initial, before storage	10.0
Temperature of storage, ° F.	
30*	6.0
40	7.0
50	7.5
60	8.8
70	10.0
80	13.3
90	16.5

* *Held for 12 hr. only.*

by a subsequent observation that freshly broken eggs of good quality had a higher fluorescence than the same material after standing at 80° F. for 16 hr. in sterile containers (see Table IV).

Similar behaviour is shown in Table III. Sera extracted from fresh Grade *A* eggs before freezing had a higher fluorescence value than sera similarly extracted from eggs candled as Grade *A* after five months commercial storage in the shell. It will be noted on examination of the standard deviations that

the variations between samples within each quality type were wide. This was not evident however where large batches of liquid egg were involved (Tables I and IV). It will also be observed in Table III that freezing consistently resulted in decreased fluorescence values.

TABLE III

MEAN FLUORESCENCE AND STANDARD DEVIATIONS FOR SERA EXTRACTED FROM EGG BEFORE AND AFTER FREEZING

(Means of 12 measurements)

Description of sample	Fluorescence			
	Before freezing		After freezing	
	Mean	Standard deviation	Mean	Standard deviation
Grade *A*, one day old	12.4	1.63	7.1	0.60
Grade *A*, stored five months at 30° F.	10.9	1.08	10.0	1.06
Grade *C*, stored two months at 30° F.	14.1	2.63	10.6	1.39
Incubator rejects, candled at four and seven days, held one week at 30° F.	17.7	2.53	15.7	1.22

Table IV shows the fluorescence values for sera obtained from Grade *A* liquid egg, from similar material after standing, and from Grade *C* and lower grade materials. The fluorescence of serum extracted from freshly broken liquid egg distinguished between quality types, Grade *C* melange being well differentiated from Grade *A*. No deterioration could be detected either organoleptically or by the fluorescence measurement in liquid egg stored 16 hr. at 80° F. in sterile glass (see also Table II).

These materials were then frozen and stored in the frozen state. Directly after freezing, the range in terms of fluorescence units was greatly narrowed—only two units separated Grade *A* and Grade *C* material. After storage at 10° F., fluorescence values for Grade *C* and cracked eggs were lowered still further. However, this drop did not appear in the materials stored at 0° and −10° F. (the temperatures used commercially) with the result that differentiation was equivalent to that for freshly frozen material. As measured by fluorescence, only minor changes in quality occurred in frozen egg stored for six months at temperatures ranging from −10° to 10° F.

Discussion

Fluorescence measurements on the serum exuded by thawing egg are considered to have distinct promise as a quality control measure in egg drying

TABLE IV

FLUORESCENCE VALUES OF SERA EXTRACTED FROM EGG MATERIALS OF VARYING QUALITY BEFORE AND AFTER FREEZING, AND AFTER STORAGE IN THE FROZEN STATE

(Means of two measurements)

Treatment	Quality of materials used						
	Fresh Grade *A** (no off-odour)	Grade *A* held 16 hr. at 80° F. in sterile glass prior to freezing (no off-odour)	Grade *C* (slight off-odour)	Cracked eggs (definite off-odour)	Musty eggs (strong off-odour)	Incubator rejects (very strong off-odour)	Mean values for treatments
Before freezing	10.0	6.3	14.0	15.0	32.0	58.2	22.6
After freezing	6.1	5.6	8.0	11.6	14.5	32.5	13.1
Three months storage at 10° F.	6.1	6.4	7.0	8.5	14.0	27.0	11.5
0° F.	6.5	7.0	8.6	8.9	14.0	26.0	11.8
−10° F.	6.7	7.2	9.4	11.8	16.4	31.0	13.8
Six months storage at 10° F.	6.3	6.5	7.0	9.7	15.9	33.0	13.1
0° F.	6.3	6.2	7.6	11.5	15.2	30.5	12.9
−10° F.	6.6	6.5	7.8	9.5	17.0	35.5	13.8
Mean values for quality types	6.8	6.5	8.7	10.8	17.4	34.2	—
Duplicate error	± 0.3			± 1.0			

* *Means of six measurements.*

plants. The fact that no serum separates from frozen egg stored for less than a month, and that 4 to 6 lb. is required for a measurement, make the measure unsuitable for most investigational purposes—but these restrictions do not apply to quality control in egg drying establishments, since the frozen egg is usually stored for more than a month before drying, and all the frozen material has to be thawed in any event before it can be used.

For investigational purposes, chloroform-extracted sera avoid expense in time and materials. This adapted procedure gives less critical results, but nevertheless appears to offer some discrimination between the quality of samples of both liquid and frozen egg. A more precise evaluation of the measure will be made in forthcoming papers of this series.

Acknowledgment

The author wishes to express her thanks to Miss B. Metcalfe for very efficient technical assistance.

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SEPARATION OF STARCH AND GLUTEN

IV. APPLICATION OF A RAPID PROCESS TO FLOURS FROM VARIOUS GRADES AND TYPES OF WHEAT[1]

By A. L. Shewfelt[2] and G. A. Adams[2]

Abstract

A rapid process for separating starch and gluten from hard wheat patent flour (consisting of dispersal of a soft dough in water followed by screening) has been applied to pastry flour, whole wheat flour, and patent flours from the following wheats: No. 1 Northern, Hard Red Spring of high protein content; No. 2 Northern, Hard Red Spring of low protein content; No. 4 Northern, Hard Red Spring, severely damaged by frost; No. 2 C. W. Garnet; No. C. W. Amber Durum; and No. 1 Alberta Red Winter. The original separation procedure required only minor modifications in spite of varying quantities and characteristics of the glutens of these flours. Whole wheat flour required a substantially greater amount of mixing water for the preparation of a satisfactory dough. Approximately 90% of the starch present in each flour was recovered. Starches from the patent flours had protein contents ranging from 0.49 to 0.64% while that from whole wheat flour contained 1.06% of protein. Recoveries of gluten from all flours were practically complete. Crude dry gluten prepared from patent flours contained 20 to 30% of starch while the bran–gluten fraction from whole wheat flour contained 9.9%.

Introduction

Most of Canada's export and domestic trade demands a hard wheat suitable for the manufacture of high quality flour. Several types and grades of Canadian grown wheat, which fail to meet this requirement becaue of unsuitable gluten quality or gluten quantity, are usually available at a lower market price and merit attention as a possible source of commercial starch.

A previous communication (1) described a rapid method for separating starch and gluten from commercial hard wheat patent flour. The present study was designed to obtain information on the application of the process to flours made from various types and grades of wheat.

Materials and Methods

Representative 1 bu. lots of each of the following wheats were used as the basis for this study.*

1. No. 1 Northern, Hard Red Spring (high protein).
2. No. 2 Northern, Hard Red Spring (low protein).
3. No. 4 Northern, Hard Red Spring (frosted).
4. No. 2 C. W. Garnet.
5. No. 1 C. W. Amber Durum.
6. No. 1 Alberta Red Winter.

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as Paper No. 36 on the Industrial Utilization of Wastes and Surpluses, and as N.R.C. No. 1371.

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* *Supplied by the Grain Research Laboratory, Board of Grain Commissioners, Winnipeg.*

After samples of each of the wheats had been reserved for analytical purposes, flours were prepared from the remaining portions by means of a Buhler experimental flour mill. In addition to these prepared flours, commercial pastry and whole wheat flour were included for comparative purposes.

After trial separations on each flour, the following conditions, judged to be most generally suitable, were selected for comparative study.

Three kilograms of flour was mixed with a fixed quantity of water at 30° C. for 15 min. For the six laboratory-prepared flours, 2550 ml. was used; for the pastry flour, 2400 ml.; and for the whole wheat flour, 3300 ml. Moisture content of the various flours was sufficiently constant to render corrections for its variation unnecessary. The dough-mixing phase was followed by a 10 min. conditioning period. The remainder of the separation and also the recovery of the products were conducted as previously described (1).

Analytical methods.—Wheat samples were ground in a Wiley mill and then ball milled for six hours. These, and samples of the various flours, recovered starches, and drum-dried glutens were analysed for starch, nitrogen, and moisture. In addition, the flours were analysed for gluten content. All methods were the same as those used in the previous study (1).

Experimental Results and Discussion

The analytical data obtained for starch, protein, and moisture contents of the various wheats and their respective flours together with flour extraction values are presented in Table I. The inverse relation of starch and protein values for the various patent flours is apparent. Starch values ranged from 65.7% for the No. 1 Northern high protein wheat flour to 70.8% for the commercial pastry flour. The corresponding protein values ranged from 14.4 to 10.6%. The analytical data for the commercial whole wheat flour were of the same order as those of the representative wheat samples.

TABLE I

ANALYSES OF THE VARIOUS WHEATS AND FLOURS USED IN COMPARATIVE SEPARATIONS

Materials	%							
	Flour extraction	Starch*		Protein*		Moisture		Gluten
		Wheat	Flour	Wheat	Flour	Wheat	Flour	Flour
High protein wheat	66	50.9	65.7	14.5	14.4	11.3	13.5	14.8
Low protein wheat	67	54.2	68.1	12.8	2.0	14.0	13.4	12.1
Frosted wheat	63	52.1	66.4	13.4	12.8	12.4	13.8	12.9
Garnet wheat	68	54.5	65.7	12.5	12.1	12.7	13.4	12.3
Durum wheat	51	54.5	66.1	14.2	13.1	12.7	13.6	12.5
Winter wheat	58	52.8	67.7	13.1	12.5	11.7	13.6	12.1
Pastry flour	—	—	70.8	—	10.6	—	13.5	9.3
Whole wheat flour	—	—	56.1	—	13.6	—	13.2	24.4**

* *Calculated on a 13.5% moisture basis.*

** *Includes bran fraction.*

Data on the recovery and purity of the starch and gluten fractions separated from the flours are given in Table II. Starch recovery was approximately 90% for all flours while gluten recovery was substantially complete. Crude starches from patent flours contained 0.49 to 0.64% of protein. Starch from

TABLE II

RECOVERY AND PURITY OF STARCH AND GLUTEN FRACTIONS SEPARATED FROM EIGHT DIFFERENT FLOURS

Type	%			
	Starch recovery	Gluten recovery	Protein content of starch*	Starch content of gluten*
High protein wheat	91	98	0.64	20.8
Low protein wheat	88	97	0.61	22.2
Frosted wheat	90	103	0.54	29.0
Garnet wheat	92	100	0.58	22.9
Durum wheat	91	98	0.54	27.1
Winter wheat	93	101	0.49	27.6
Pastry	88	99	0.51	22.9
Whole wheat	90	98	1.06	9.9

* *Air-dry basis.*

whole wheat flour contained 1.06% of protein. Starch contents of the crude dry glutens ranged from 20 to 30%, while that of the bran–gluten fraction from whole wheat flour was 9.9%. Thus, despite the wide range in patent flour source, the variation in amounts and purity of starch and gluten was comparatively small. Some of the variation can be explained by differences in the water-absorptive capacities of the flour, and, in practice, could be compensated for by adjusting the amount of dough-mixing water for each type of flour.

Since whole wheat flour had a higher water-absorptive capacity than the patent flours, 3300 ml. of mixing water for each 3 kgm. of flour (13.6% moisture) was required to produce a satisfactory dough. Starch obtained from whole wheat flour was somewhat dark in colour, and, while suitable as a fermentation substrate, it would require considerable refinement in order to meet commercial starch requirements. The starch content of the bran–gluten fraction was lower than that of the glutens but since this fraction represented over 25% of the original flour, the proportion of total starch which it contained was of the same order as for the other flours.

Data on the distribution of starch and protein in the separated fractions were calculated and are presented in Table III. Again the variation was comparatively small except for the proportion of total protein in the gluten fraction which varied from 76.6 to 87.6%. This variation was attributed to differences in the proportion of soluble proteins within the various flours.

TABLE II

DISTRIBUTION OF STARCH AND PROTEIN IN FRACTIONS SEPARATED FROM FLOURS

Type	Proportion of total starch, %		Proportion of total protein, %	
	Starch fraction	Gluten fraction	Starch fraction	Gluten fraction
High protein wheat	91.0	6.3	3.0	87.6
Low protein wheat	88.2	5.4	3.6	81.9
Frosted wheat	90.4	9.1	3.0	85.7
Garnet wheat	91.8	6.0	3.5	87.1
Durum wheat	91.3	5.0	2.9	82.1
Winter wheat	92.9	7.5	3.1	87.1
Pastry	87.6	4.1	3.5	76.6
Whole wheat	89.6	4.5	4.7	78.9

From the above results, it can be stated that this separation process can be applied satisfactorily to flours from many grades and types of wheat, e.g., pastry flour with a protein content as low as 10.6% was entirely satisfactory. Low protein wheat has an advantage over high protein wheat because of its higher starch content and, as a general rule, its lower market value.

The variations in gluten characteristics of the various flours had little effect on the separation. It was noted that the gluten from Durum wheat flour had a soft fluid texture and was feathery when drum-dried. With whole wheat flour separations, complete removal of the bran from the gluten was difficult and in practice the two would probably remain combined and be dried as a feed product.

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THE THIAMIN CONTENT OF MEAT[1]

RITA CAMPBELL[2], MARY C. HILTZ[3], AND ALLEN D. ROBINSON[4]

Abstract

Meats purchased in Winnipeg retail stores contained less thiamin than is reported in the literature for the same meats from other places. The values for pork compare with the ones reported in the literature, but those for other meats are distinctly lower. Arranged in descending order of thiamin content, the meats studied were: pork, variety meats, fowl, lamb, veal, and beef. Losses in cooking are quite high for fowl and some variety meats. Stewing and parboiling cause greater losses than roasting or frying.

The importance of thiamin or vitamin B_1 in our diets is too well understood to need emphasis. Among its better sources is meat—particularly pork, but also the liver, organs, and muscles of many animals. A publication of the Ministry of Food, London (10), reveals that, prior to the war, meats, as carcass weight, comprised about 12% of the food available to each Canadian consumer. By 1943 the total consumption of food per capita had increased, as had that of meat, the latter being about 13% of the total. Meat must, then, supply an appreciable part of the thiamin we consume.

There have been a number of reports published on the thiamin content of various meats. One of the most comprehensive is that of Booher, Hortzler, and Hewston (1). The following tabulation indicates some of the results reported by different workers, these being expressed on a wet basis as micrograms per gram of raw meat, except where otherwise indicated.

Meat	Hiltz *et al.* (5)	Waisman and Elvehjem (12)	McIntire *et al.* (7, 8)	Miller *et al* (9)	Lane *et al.* (6)	Reedman and Buckley (11)	Cover *et al* (3)	Booher *et al.* (1)
Pork loin chops	5.34–8.67	—	—	—	—	—	—	13.7–14.6
Pork loin roasts	6.10–7.78	11.1	7.4–15.2	9.2–27.8	—	—	—	—
Pork shoulder roasts	4.54–5.33	—	—	7.1–21.1	—	—	—	—
Ham	5.52–8.20	9.1	7.7–14.8	8.8–29.9	11.8	—	—	15.3–30.0
Luncheon meat	2.26–4.71	—	—	—	—	9.6–20.7*	—	—
Bacon	3.51–5.26	—	1.9	—	—	—	—	—
Chicken	—	—	—	—	—	—	—	0.8
Lamb	—	—	—	—	—	—	—	4.36
Beef	—	—	—	—	—	—	1.2–1.4	—
Veal	—	—	—	—	—	—	—	1.2– 4.3
Pork liver	—	4.4	—	1.9– 7.3	—	—	—	15
Beef liver	—	—	2.3	—	—	—	—	1.8– 3.9
Baby beef liver	—	—	1.9	—	—	—	—	—
Veal heart	—	—	7.0	—	—	—	—	—
Beef kidney	—	—	—	—	—	—	—	3.16

* *Moisture-free basis.*

[1] *Manuscript received November 5, 1945.*

Contribution from the Departments of Foods and Nutrition and of Chemistry of The University of Manitoba, Winnipeg, Man., with financial assistance from the Swift Canadian Co., Ltd.

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When meat is cooked some of the thiamin in it is destroyed and so is lost to the consumer. McIntire, Schweigert, Henderson, and Elvehjem (7) found that cooking losses for pork varied with the cooking method. Braising losses in lean meat were greater than those for roasting or broiling, though the total retention of thiamin in meat and drippings was the same, irrespective of the cooking method. Waisman and Elvehjem (12) report losses as great as 50, 55, and 56% in roasting pork, veal, and beef, respectively. That frying losses were less, they suggest, may be due to the shorter cooking time required. Stewed kidney and heart of beef showed losses of 40 and 57%. Hiltz, Robinson, and Levinson (5) found cooking losses for pork to vary from 7 to over 80%. Roasting caused losses of approximately 20% in shoulder butt roasts and 40% in ham. Frying pork chops caused losses up to 38%. Extremely high losses occurred when bacon was fried. Cover, McLaren, and Pearson (3) found thiamin retention in rare and well done rib roasts of beef to be 75 and 69%, respectively.

It was decided to investigate further the amounts of thiamin in meat by analysing meat purchased in Winnipeg retail stores. This would provide additional data to that available in the literature. It would indicate, too, whether the amounts of thiamin in meat sold in this locality are the same as those in meat sold elsewhere. Differences might be expected, since animals in different areas are often provided with different kinds of feed. It was decided, too, to investigate cooking losses of thiamin for the meat studied.

Experimental

The meats used in this investigation were beef, veal, lamb, pork, variety meats, and poultry. Standard household cuts were purchased for uncooked analysis. Similar adjacent cuts were purchased for cooking studies. Fowl, pork and beef liver, and lamb and beef kidneys were divided in halves and a half of each assayed uncooked and a half used for cooking studies. Separate baby beef hearts were used for assays of uncooked and cooked meats. The white and dark meat of fowl were analysed separately.

Each cooked cut was weighed and divided into meat, bone, visible fat, and drippings, which were weighed separately. The meat alone was retained for analysis, since in previous investigations reported by Hiltz, Robinson, and Levinson (5) it had been found that the amount of thiamin in dripping is negligible. The meat was ground in a meat grinder to make it homogeneous. Samples were taken for duplicate thiamin assays and for duplicate moisture tests. In the analysis of raw samples, meat was removed from the bone and weighed, as were the bone and visible fat portions. The meat was put through a meat chopper twice. Duplicate samples were taken for thiamin and moisture assays.

Meats were cooked following standard methods of cookery for general household usage as outlined by the Committee on Preparation Factors, National Cooperative Meat Investigators (2). Oven temperatures, internal temperatures—as shown by a Taylor meat thermometer at the centre of the

roast—and cooking times were noted. The cooking of roast meats was done at specified oven temperatures until the required internal temperature was reached. Where the use of a meat thermometer was not feasible, meats were cooked a recommended time as specified in the above reference, the time being varied according to the weight of the cut. Beef steaks and veal cutlets, pork and lamb chops were pan broiled in a preheated skillet to a well done stage. Stewing beef from the neck was simmered in water to cover until tender. The cooking of variety meats is described in Table V. A 5 gm. portion of lard was used in broiling pork liver. Lamb and beef kidney were soaked in cold water for one hour before parboiling.

Thiamin was determined by a modification of the Hennessy and Cerecedo (4) method used in a previous investigation by Hiltz, Robinson, and Levinson (5). The method was checked with two outside standards, commercial Winthrop's Vitamin B_1 Standard, and a thiamin solution of known strength provided by Dr. Hoffer of Western Canada Flour Mills Co., Ltd., of Winnipeg. Moisture was determined by the vacuum-oven method.

The experimental data are presented in Tables I to V, inclusive. These show thiamin content and cooking losses for beef and veal, pork, lamb, fowl, and variety meats. Each figure represents the average of duplicate determinations made on the edible portion. The values are higher than if they

TABLE I

THIAMIN CONTENT OF RAW AND COOKED BEEF AND VEAL

Meat	Thiamin (wet basis), μgm./gm.		Cooking treatment			Thiamin lost in cooking, %
	Uncooked	Cooked	Internal temp., °F.	Oven temp., °F.	Cooking time, min.	
Rump roast	0.20	0.30	165	315–25	129	—
Prime rib roast	0.24	0.29	165	315–25	102	10.2
Round bone shoulder roast	0.19	0.23	165	315–25	94	5.5
Porterhouse steak	0.21	0.30	—	—	12	1.0
Blade roast	0.20	0.29	165	315–25	102	1.3
Sirloin steak	0.30	0.39	—	—	18	13.1
Wing roast	0.21	0.32	165	315–25	116	—
Round steak	0.28	0.33	—	—	19	19.9
Neck stewing meat	0.44	0.40	—	—	92	74.8
Veal cutlets	0.51	0.27	—	—	14	67.1
Veal round steak	0.50	0.82	170	310–20	124	—

had been based on the inedible part of the meat as well. Because the thiamin contents are small they are expressed to hundredths of a microgram, rather than to tenths as is usually done. Percentage loss was calculated from thiamin contents reduced to a dry basis. Essential cooking data—time, oven temperature, and internal temperature—are included in the tables.

TABLE II

THIAMIN CONTENT OF RAW AND COOKED PORK

Meat	Thiamin (wet basis), μgm./gm.		Cooking time, min.	Thiamin lost in cooking, %
	Uncooked	Cooked		
Loin roast No. 1	6.15	6.46	85	18.4
Loin roast No. 2	7.79	8.36	108	23.3
Chop No. 1	9.64	7.16	10	58.5
Chop No. 2	8.10	9.01	9	22.1
Chop No. 3	7.33	8.69	7	11.1
Chop No. 4	7.52	9.63	7	17.0
Chop No. 5	10.04	12.32	9	24.0

Roasts were cooked to an internal temperature of 182° F. in an oven whose temperature ranged from 310 to 350° F.

TABLE III

THIAMIN CONTENT OF RAW AND COOKED LAMB

Meat	Thiamin (wet basis), μgm./gm.		Cooking time, min.	Thiamin lost in cooking, %
	Uncooked	Cooked		
Hind leg lower portion	0.50	0.57	113	22.4
Hind leg upper shank	0.36	0.42	130	11.7
Neck roast	0.18	0.19	127	31.5
Breast and shank	0.21	0.37	112	—
First rib chop	0.34	0.44	11	—
Last rib chop	0.36	0.44	10	10.4
Loin chop No. 1	0.63	0.74	10	15.8
Loin chop No. 2	1.18	1.30	12	23.4

Roasts were cooked to an internal temperature of 175° F. in an oven whose temperature ranged from 310 to 320° F.

Discussion

The values obtained for the thiamin content of beef vary from 0.19 to 0.44 μgm. per gm. of raw meat. This is somewhat lower than those reported by Cover *et al.* (3), which are from 1.2 to 1.4. Cooking losses are not very large except for stewing beef. Here a large part of the thiamin may have been extracted by the cooking water. Other beef cuts show losses up to 20%. The amounts of thiamin found in veal, 0.50 and 0.51 μgm. per gm., are lower than those reported by Booher, Hortzler, and Hewston (1). The data on cooking losses are not sufficient to justify drawing conclusions at this time. The two cuts of veal examined contained more thiamin than beef but could not be considered good sources of the vitamin.

The thiamin content of pork roasts as shown in Table II is comparable with the values reported in the literature. The values for chops are of the same order as those for roasts. Cooking losses for roasts are 18.4 and 23.3%, while for chops they are from 11.1 to 24.0%, with one chop showing a loss of 58.5%.

TABLE IV

THIAMIN CONTENT OF RAW AND COOKED FOWL

Meat	Thiamin (wet basis), μgm./gm.		Cooking treatment		Thiamin lost in cooking, %
	Uncooked	Cooked	Oven temp., °F.	Cooking time, min.	
Chicken broiler			380	22	
Light meat	0.26	0.36			—
Dark meat	0.49	0.47			78.1
Liver	2.02	—			—
Roast chicken			325–50	80	
Light meat	0.44	0.36			40.3
Dark meat	0.63	0.46			49.3
Liver	—	2.03			—
Stewing fowl			—	155	
Light meat	0.21	0.06			78.0
Dark meat	0.27	0.11			74.1
Cooking liquid	—	0.03			—
Roast turkey			350	105	
Light meat	0.27	0.25			26.2
Dark meat	0.38	0.36			37.1
Liver	—	1.06			—

TABLE V

THIAMIN CONTENT OF RAW AND COOKED VARIETY MEATS

Meat	Thiamin (wet basis), μgm./gm.		Cooking treatment	Thiamin lost in cooking, %
	Uncooked	Cooked		
Pork liver	1.19	1.32	Broiled 20 min.	21.4
Beef liver	1.05	1.24	Parboiled 15 min.	14.5
Lamb kidney	1.49	1.87	Parboiled 10 min.	52.1
Beef kidney	1.98	2.08	Parboiled 10 min.	46.0
Baby beef hearts	1.90	1.61	Baked 80 min.	52.0
	—	1.87	Boiled 105 min.	63.0

Thiamin values for cuts of lamb assayed are presented in Table III. They are from 0.18 to 1.18 μgm. per gm. These are lower than those reported by Booher *et al.* (1). Cooking losses for roasts and chops varied from 10.4 to 31.5%.

The thiamin content of fowl as shown in Table IV is less than that reported by Booher *et al.* The amount of thiamin in dark meat is higher than in white. This has been reported by Waisman and Elvehjem (12). Cooking losses are quite high, especially in stewing fowl. The data show that a part of this thiamin was not destroyed but was extracted by the cooking liquid.

The variety meats listed in Table V contain appreciable amounts of thiamin, but less than those reported by McIntire, Schweigert, Herbst, and Elvehjem (8). Cooking losses for livers were 14.5 and 21.4%. Those for kidneys and baby beef hearts were quite high, being from 46.0 to 63.0%.

While the number of samples tested was not sufficient to permit one to draw hard and fast conclusions, the results do indicate that, with the possible exception of pork, meats sold in Winnipeg retail stores contain less thiamin than has been reported for meat elsewhere. The order of decreasing thiamin contents is: pork, variety meats, fowl, lamb, veal, and beef. This is the same order as results reported elsewhere, save for fowl which other investigations place below beef.

Cooking losses for most meats are less than 25%. Exceptions noted include fowl and variety meats. A comparison of cooking temperatures and times with losses reveals no apparent relation to those factors. A more important cause of loss seems to be the use of water in the cooking process, as in the stewing of beef and fowl and the boiling and parboiling of variety meats. This extracts the thiamin from the meat. It is not destroyed completely, but is available in part in the cooking water.

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RESIN–RUBBER FROM CANADIAN GROWN PLANTS

V. METHODS OF EXTRACTION FROM POD HULLS OF THE COMMON MILKWEED[1]

By R. V. Tomkins[2] and N. H. Grace[3]

Abstract

Yields of 2 to 3% of a resin–rubber gum from milkweed pod hulls were obtained by a process that included alkaline digestion and pebble milling. The effects of various digestive treatments and milling procedures in laboratory and pilot plant are reported. The pod hulls contained 10% of resin–rubber, but 40% of this was lost during digestion. However, the resin–rubber content of the digested material was 17%. The pebble mill comminuted the plant tissue and agglomerated the resin–rubber, the milling conditions having a marked effect on the efficiency of this operation.

Introduction

Seed pod hulls of the common milkweed, *Asclepias syriaca* L., are available from the mechanical separation of floss that is being produced in large quantities as a substitute for kapok. Since the unopened pods contain approximately equal amounts of floss, seeds, and hulls, the available quantity of hulls equals the weight of floss that is produced. Consequently, a substantial bulk of hulls is available and the amount will probably increase if the floss industry is maintained in the post-war period. Economic utilization of the pod hulls would assist this new industry to meet the inevitable competition from kapok.

Consideration has recently been given to milkweed as a source of a mechanically extracted resin–rubber gum (2). This extraction process has been carried to pilot plant scale using milkweed leaves as raw material (3). The extraction of resin–rubber from pod hulls on a laboratory scale has also been reported (2). This communication describes both laboratory and pilot plant studies of the processes involved in the extraction of resin–rubber from the pod hulls.

Materials and General Methods

Materials and Equipment

Pod hulls obtained from Petosky*, Michigan, served as the raw material for the extraction reported herein. Eight samples, collected at random, were analysed. The following average values were obtained: moisture 8.6%, resin–rubber 9.85%, and residue 81.55%. These analytical values were

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* *Baled pod hulls were kindly supplied by the Hemp Division, Commodity Credit Corporation, Petosky, Mich. These hulls from the mechanical extraction of floss contained approximately 10% of floss and seeds.*

used for all calculations except in the study of digestion conditions in the laboratory.

The alkali used throughout this investigation was commercial flake sodium hydroxide.

Laboratory and pilot plant studies utilized steam-heated digestion equipment, cheesecloth filters, porcelain-lined pebble mills, and vibrating stainless steel screens, which have been described previously (2, 3). The laboratory mill had a gross capacity of 55 litres and an internal diameter of 16 in. The pebble charge occupied 25% of the gross volume and the slurry charge, 33%, leaving 42% free space. Flint pebbles, average diameter 1.1 in., were used. The larger scale pilot plant mill had an internal diameter of 33 in. and a gross capacity of 140 gal. The pebble and slurry charges were proportioned as in the small mill, but larger pebbles, average diameter 2.3 in., were used. From information on the grinding of minerals, 80% of theoretical critical speed was selected for maximum capacity (1, pp. 29-31). Rotation of the laboratory mill at 53 r.p.m. attained this value, but the larger pilot plant mill was held to 70% (32 r.p.m.) to avoid excessive wear on the porcelain lining.

Procedure

The general method involved digestion, washing to the required pH on the filter, pebble milling followed by screening, and return of the oversize to the mill for final agglomeration.

Analysis of Pod Hulls and Resin–rubber Gums

The analytical procedures have been described (4). The rubber content of the mechanically extracted resin–rubber is low, and error in its determination results if the acetone extraction is not complete. However, the total resin and rubber obtained by successive 24-hr. extractions with acetone and benzene is relatively constant, and this value, designated "resin–rubber," is used throughout this communication. The remainder, insoluble in acetone and benzene, is termed "residue." Percentage yields are reported on a basis of dry resin–rubber, and air-dry pod hulls.

Experimental

In a preliminary series of laboratory extractions of pod hulls, alkali concentration, temperature, and time of digestion were varied but milling conditions were maintained constant. The results showed great irregularity in yield from duplicates. No resin–rubber was agglomerated from material digested in water or 0.5% alkali. Subsequently, laboratory studies were designed to elucidate sources of gross variability and to obtain data useful in scaling up the process to the pilot plant.

Application of laboratory procedures, with only minor modifications, to pilot plant scale extractions resulted in successful agglomeration of the resin–rubber. Pilot plant experiments were performed to gather information on digestion and milling conditions from which economically optimum procedures might be selected.

Digestion

Alkaline digestion was used as a means of releasing resin–rubber from the plant tissue and to effect concentration of the resin–rubber by removing part of the residual material. As this treatment also removed some resin–rubber, the effects of alkali concentration, temperature, and duration of digestion were investigated to determine which conditions led to maximum concentration of resin–rubber with minimum loss of this desired fraction.

A 7 kgm. mass of pod hulls was mixed, sampled, and analysed. The material contained 6.2% of moisture, 9.6% of resin–rubber, and 84.2% residue. (The resin–rubber content on a moisture-free basis was 10.3%.) Batches of hulls (250 gm.) were digested, the ratio of solution to pod hulls being maintained at 15 : 1. From previous work (2), basic digestion conditions of two hours at 121° C. in 1.5% alkali were selected. The three factors were varied individually around this basis over the ranges indicated in Figs. 1 and 2. In addition, the effect of a 15 min. water extraction at 100° C. prior to digestion was investigated. The duration of the treatment was measured from the time the charge reached the desired temperature. The digested hulls were washed to pH 9.5 and moistures determined on the total drained mass to avoid sampling errors. Analysis of the dried material permitted calculation of loss of resin–rubber and residue resulting from various digestive treatments.

Eight 1-kgm. samples of pods were taken at random, analysed, and digested. The resin–rubber contents before and after digestion are compared in Table I. It is apparent that the precision of results after digestion is comparable to the variability of the source material.

TABLE I

VARIANCE IN REPLICATE SAMPLES BEFORE AND AFTER DIGESTION

Digestion conditions: two hours at 100° C. in 1.5% alkali

	Before digestion		After digestion	
	Resin–rubber	Residue	Resin–rubber	Residue
Gm. per kgm. air-dry hulls	98.5	815.5	59.4	306.0
Standard deviation, gm.	7.4	15.8	4.46	8.9
Coefficient of variability, %	7.50	1.94	7.51	2.91

The reduction of the residue content of the pod hulls during digestion and the accompanying loss of resin–rubber are shown in Fig. 1. Digestions at lower alkalinity and temperatures, and for shorter times, resulted in relatively greater loss of residue than of resin–rubber. Loss of resin–rubber increased as the treatment became more drastic, until finally a greater percentage of this fraction was lost. Water digestion before the alkali treatment had little effect. The flatness of the curves describing temperature and time

effects is probably due to the alkali concentration (1.5%) at which these trials were performed. The losses were not entirely due to chemical action, as small particles were lost during filtration.

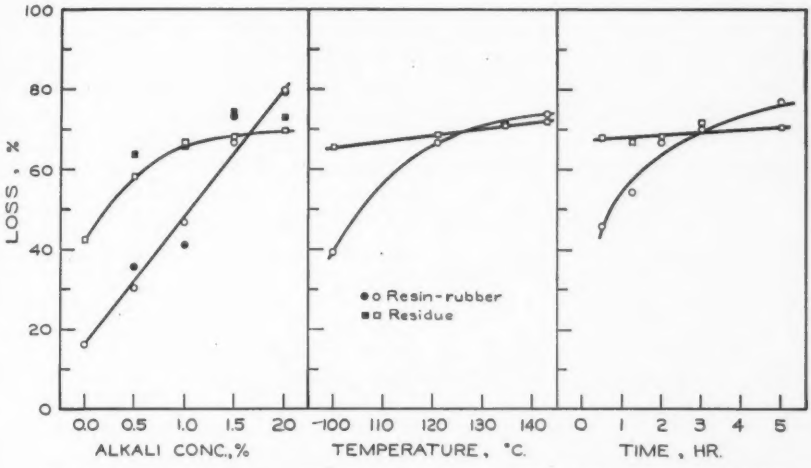

FIG. 1. *Effect of digestion conditions on loss of resin–rubber and residue. Solid points indicate prior water extraction.*

Residual resin–rubber in the digested pod hulls is shown in Fig. 2. As the intensity of treatment increased, the resin–rubber content of the digested material rose to a maximum, then fell below that of the undigested hulls

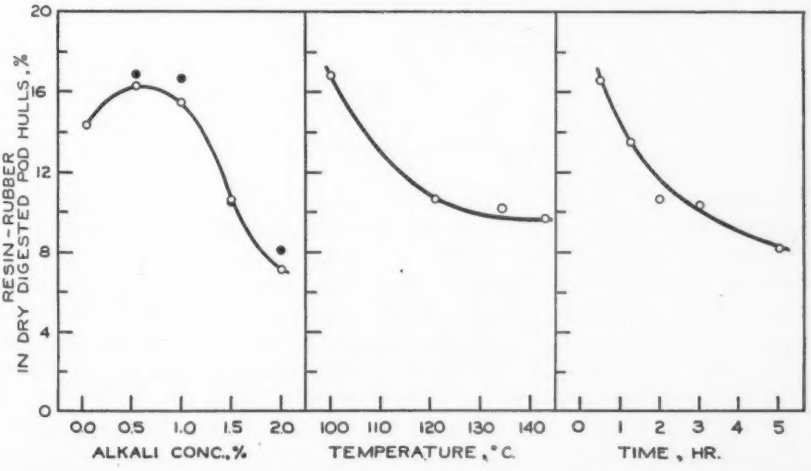

FIG. 2. *Effect of digestion conditions on concentration of resin–rubber after digestion. Solid points indicate prior water extraction.*

(10.3%). Over the range investigated, alkali concentration had most marked effect, and the use of lower temperatures and a short cooking time is indicated.

The dependent variables are plotted in Figs. 3 and 4 to show the continuity of effects of cooking treatments. Fig. 3 indicates that the loss of residue approaches a limiting value at about 72%, whereas resin–rubber loss shows no such limit. The curve in Fig. 4 shows a pronounced maximum at 17% of resin–rubber in the digested material. Obviously, digestion conditions in the vicinity of the maximum are most desirable, as more drastic treatment resulted not only in further loss of the desired fraction but also in failure to

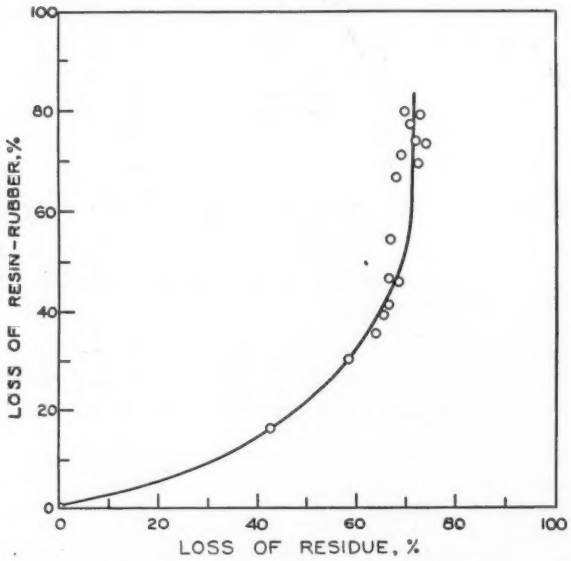

FIG. 3. *Relation between loss of resin–rubber and loss of residue during digestion.*

increase the resin–rubber content of the digested hulls. The points near the maximum correspond to the following digestion conditions: 2 hr. at 121° C. in 0.5 and 1.0% alkali; 2 hr. at 100° C. in 1.5% alkali; and 0.5 hr. at 121° C. in 1.5% alkali. Material digested in 0.5% alkali yielded no resin–rubber when milled. Although the resin–rubber content of the mill charge was high, apparently digestion did not release the resin–rubber from the plant tissue sufficiently for successful agglomeration. The remaining three sets of conditions were selected, therefore, for pilot plant trials.

Duplicate millings of hulls digested as indicated above were performed in the pilot plant. The cooked material was washed to pH 9.5, milled at 3.6% solids for three hours, screened, and the oversize remilled with wash water for 1.5 and 0.5 hr. The yields are compared in Table II. Hulls digested

in 1.0% alkali did not mill as well as those obtained by the other digestive treatments. Subsequent milling experiments made use of pod hulls digested for two hours at 100° C. in 1.5% alkali.

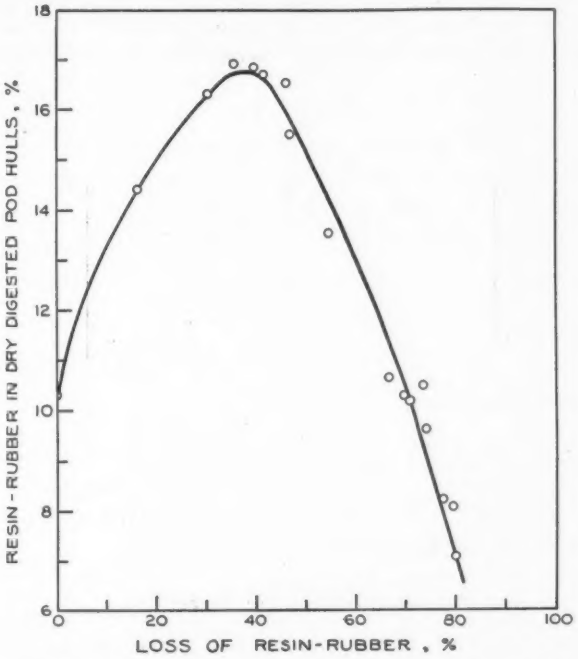

FIG. 4. *Relation between concentration of resin–rubber after digestion and loss of resin–rubber during digestion.*

TABLE II

YIELDS OF RESIN–RUBBER AFTER VARIOUS DIGESTIVE TREATMENTS

Digestion			Yield of resin–rubber, %
Duration, hr.	Temperature, ° C.	Alkali conc., %	
2	121	1.0	1.1
2	100	1.5	1.7
0.5	121	1.5	1.7

Washing

Removal of dissolved substances and excess alkali was accomplished by draining and washing the digested pods with warm water. Two-kilogram samples of pod hulls were digested at 100° C. for two hours in 1.5% alkali and washed to different pH values. Subsequently, duplicate millings of four

hours at 1.8% solids and loading temperature of 45° C. were performed, and the yields of resin–rubber recorded. Sulphuric acid was added at a late stage of washing to obtain material with pH less than 7.

The data in Table III indicate a maximum yield as the pH approaches 7, with low yield from both strongly acid or alkaline material. The low yields were probably due to the reaction of residual alkali or acid with the resin

TABLE III

EFFECT OF pH ON YIELD OF RESIN–RUBBER

pH of digested, washed sludge	Yield of resin–rubber, %	pH of digested, washed sludge	Yield of resin–rubber, %
11.8	0.3	7.6	3.1
10.1	2.2	6.3*	1.5
9.5	2.4	4.5*	1.1
8.3	2.5	3.0*	0.8

* *Acid added after washing.*

fraction during milling. A pH of about 9 was readily attained with little water and labour, but greatly increasing amounts of both were required to reduce this appreciably. More thorough washing would have to be justified by a balance of additional yield against added cost.

Pebble Milling and Screening

The digested, washed pod hulls were pebble milled and the agglomerated resin–rubber concentrated by screening. Gelatinization of the cellulose and emulsification of the resin–rubber must be minimized if successful separation of the desired fraction is to be attained. Since the effectiveness of the milling procedure was judged by the amount retained on the screen, the importance of the screening operation is obvious. Indeed, the wide variation in yield for duplicates in preliminary work was traced to the use of a 12 mesh screen, which allowed varying amounts of resin–rubber to pass. A microscopic study of the particles passing the 12 mesh screen indicated that 80% of the resin–rubber would be retained on 60 mesh. The adoption of this screen eliminated the variation mentioned, but required the use of an additional 30 min. washing period to agglomerate the resin–rubber, as a greater portion of the residue was also retained. The effects of slurry consistency and temperature on the yield and the effects of duration of milling and screen size on the efficiency of the screening operation were investigated in the laboratory.

The percentage of resin–rubber and residue retained on 20, 60, and 100 mesh screens after three, four, and five hours' milling is shown graphically in Fig. 5. The use of 100 mesh slightly increases resin–rubber retained but is offset by excessive amounts of residue and the extra time required for

screening. A 20 mesh screen is effective for separation of the fractions but retains only slightly more than half the resin–rubber present in the slurry. Screening on 60 or 100 mesh after three hours' milling does not effectively separate the two components. Extending the milling period to

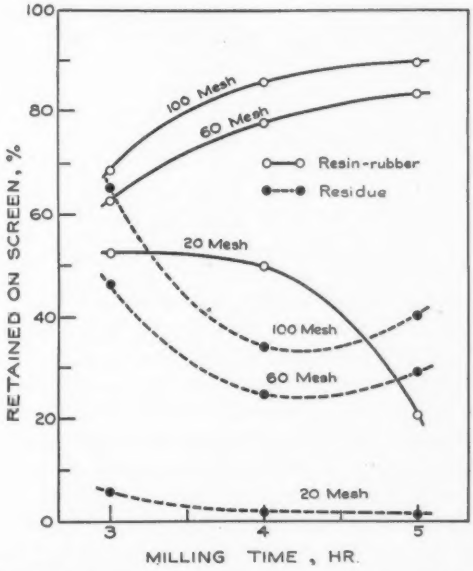

FIG. 5. *Separation of resin–rubber and residue by 20, 60, and 100 mesh screens after milling three, four, and five hours.*

five hours results in a noticeable gelatinization or swelling of the residue, causing more of this undesirable material to be retained than after four hours' milling. The separation at four hours on 60 mesh appears most satisfactory.

The effects of slurry consistency and slurry temperature are shown in Table IV. At the higher consistencies, the slurry became excessively thick and difficult to handle, with reduced yields. Solid contents lower than 2.4% not only reduced the capacity of the mill (i.e., less resin–rubber per charge) but the percentage yield fell off. The slurry temperature had little effect on the yield except at 5° C.

The change in grinding characteristics accompanying increase of mill and pebble size necessitated a restudy of conditions of slurry consistency and duration of milling. Consideration of the heavier stones and longer falls in the large mill suggested the possibility of successful agglomeration using thicker slurries and shorter milling times than were required under laboratory conditions.

TABLE IV

EFFECT OF SLURRY CONSISTENCY AND SLURRY TEMPERATURE ON YIELD OF RESIN–RUBBER (LABORATORY)

Milled four hours, screened on 60 mesh

Slurry temp., loading, ° C.	Slurry consistency, % solids	Yield resin–rubber, %
45	1.2	1.1
45	1.8	2.4
45	2.4	3.0
45	3.0	0.9
5	1.8	1.6
25	1.8	2.5
45	1.8	2.4
65	1.8	2.5

The resin–rubber from preliminary pilot plant millings was excessively sticky and difficult to handle, but charging the mill with cold water (5 to 10° C.) eliminated this trouble. After the main milling period the ground hulls were passed over a 60 mesh vibrating screen and the oversize returned to the pebble mill with fresh water and milled an additional one and one-half hours. The resin–rubber particles were agglomerated to pieces about 1 in. in diameter by this time. The charge was drained through a coarse screen to retain the pieces of resin–rubber, and the oversize was again returned to the mill with water for a half-hour final agglomeration period. At the end of this period, the resin–rubber was removed as one or two large pieces.

The yields of resin–rubber obtained under various milling conditions are given in Table V, as pounds recovered per milling, percentage yield, and time-rate of yield. In batch milling the yield per milling is of great importance, but, were the process to be made continuous, the time rate of yield would govern conditions to be selected. A pronounced maximum is attained with four hours' milling at 3.6% solids, but it is possible that a maximum for each slurry consistency would occur at a different time. However, above 3.6% solids, the slurry becomes excessively viscous, entailing screening difficulties, while below this value the capacity of the mill per charge decreases in a batch process. Extending the period of milling beyond five hours, as previously mentioned, results in gelatinzation or swelling of the cellulose, which hinders agglomeration.

The crude resin–rubber removed from the pilot plant mill was remarkably consistent as to proximate composition, averaging 40% of water, 5% of residue (largely silica from the mill lining), and 55% of resin–rubber. The chemical nature of the resin fraction has been reported (5). The resin–rubber may be stored for short periods with little decomposition under flowing water changing every four or five hours.

The yields obtained in the pilot plant were not as high as in the laboratory, probably owing to the more violent action in the pebble mill causing a greater

TABLE V

EFFECT OF SLURRY CONSISTENCY AND DURATION OF MILLING ON YIELD OF RESIN–RUBBER (PILOT PLANT)

All charges digested two hours at 100° C., 1.5% alkali

Milling time, hr.	Slurry consistency, % solids	Resin–rubber charged to mill*, lb.	Resin–rubber recovered, lb.	Over-all yield resin–rubber, %	Yield rate, lb./hr. milling**
4	1.8	1.18	0.21	1.1	0.03
4	2.7	1.77	0.36	1.2	0.06
4	3.6	2.36	0.94	2.3	0.16
4	5.4	3.54	0.78	1.3	0.13
2	3.6	2.36	0.52	1.3	0.13
3	3.6	2.36	0.69	1.7	0.14
4	3.6	2.36	0.94	2.3	0.16
5	3.6	2.36	0.53	1.3	0.08
6	3.6	2.36	0.00	0.0	0.00

* *Based on analysis of digested hulls.*
** *Milling time plus 2 hours' washing.*

amount of emulsification of the resin–rubber. The milling operation is extremely complex, and caution should be observed in interpreting the data, as they are valid only under carefully defined conditions and with specific equipment.

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